INTROGRESSING RESISTANCE TO *FUSARIUM* ROOT ROT IN SELECTED FARMER PREFERRED ANDEAN BEAN GENOTYPES FROM MESO-AMERICAN LINE AND MAPPING OF ASSOCIATED RESISTANCE QUANTITATIVE TRAIT LOCI

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DECLARATION

I hereby declare that this thesis is my original work and has not been presented for a degree in any other University.

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SUMMARY

The common bean (*Phaseolus vulgaris* L.) is a primary protein source in the diet of many low-income populations worldwide. Bean root rots have been reported to occur in most bean fields throughout the world. In Uganda, bean root rot is one of the major constraints to bean production, with that caused by *Fusarium solani* (Mart.) f.sp. *phaseoli* (Burkholder) (N.C. Snyder & H.N. Hans) resulting in substantial yield losses. The use of resistant varieties is probably the most effective control measure against *Fusarium* root rot, especially for small-scale farmers with limited access to fungicides. Sources of resistance to *Fusarium* root rot exist in common beans and have been reported to occur in Africa. Most of the developed and identified resistant genotypes are either late-maturing and small or black-seeded, with a climbing growth habit. None of the commercial Andean bean varieties currently grown in Uganda are resistant to this pathogen. Genetic resistance to *Fusarium* root rot is polygenic and is strongly influenced by environmental factors. Response to selection for quantitative traits, such as root rot resistance, is slow due to the genetic complexity of the trait and the difficulty in evaluating resistance. Indirect selection for *Fusarium* root rot resistance based on genetic markers linked to the quantitative trait loci (QTL) for resistance would facilitate improvement, given the limitations of field selection, which are expensive, not consistent across environments and require destructive sampling. The overall objective of this study was to develop approaches based on quantitative trait loci for improving resistance in common beans to *Fusarium* root rot. The specific objectives were: (i) to confirm the usefulness of a Meso-american source (MLB 49-89A) in transferring resistance to *Fusarium solani* f.sp. *phaseoli* into locally adapted Andean types (K20 and K132); (ii) to identify quantitative trait loci that condition resistance to *Fusarium* root rot in common beans.

Two populations of 90 and 78 $F_{4:5}$ recombinant lines from K20 x MLB-49-89A and K132 x MLB-49-89A respectively, were used to confirm the usefulness of a Meso-american source (MLB 49-89A) in transferring resistance to *Fusarium solani* f.sp. *phaseoli* into locally adapted Andean types (K20 and K132). Both K20 and K132 are susceptible to *Fusarium* root rot, while MLB-49-89A is resistant. The two populations and their parents were evaluated for *Fusarium* root rot in a screenhouse using a randomised complete block design with two replications in wooden trays measuring 0.74 x 0.42 x 0.115 m. The K20 x MLB-49-89A population was skewed toward resistance while K132 x MLB-49-89A was skewed toward susceptibility. These results therefore clearly show differences in parental effects of K20 and K132 on the resistance to *Fusarium* root rot. Such differences in means and distributions between the two populations suggest that K20 possesses one or more genes that interact in an epistatic manner with two or more resistance loci in MLB-49-89A. In contrast, K132 apparently lacks the beneficial allele that is present in K20, or has an alternate allele that enhances susceptibility. The frequency distributions for both populations were bimodal, suggesting that a major gene was involved in resistance. A number of lines that had very good resistance levels to *Fusarium* root rot were identified from both populations. Both the narrow and broad sense heritabilities obtained for K132 x MLB-48-89A in this study were very high (h^2_B=0.99; h^2_N=0.98, referenced to additive variance in the F_2, reported on a line-mean basis from 2 replications). On the same basis, heritability estimates in K20 x MLB-48-89A were also high (h^2_B=0.86; h^2_N=0.81). The heritability estimates obtained in this study and in previous studies by Mukankusi (2007) indicate that improvement of the Andean varieties for resistance to *Fusarium* root rot using the Meso-american line MLB-49-89A should be possible. The results of this study have shown that when when using MLB-49-89A, it would be easier to improve K20 than K132 for *Fusarium* root rot resistance.

The cross of K132 x MLB 49-89A was emphasized for the mapping study. A total of 35 SSR markers were screened for polymorphism in the parents. Fifteen of the 35 SSR markers were polymorphic, representing 42% of the tested SSR markers. Twelve of the SSR markers gave clearly distinguishable bands and were therefore used for analysis. A mapping population of 62 $F_{4:5}$ recombinant inbred lines of K132 x MLB 49-89A was used for identifying quantitative trait loci conditioning resistance to *Fusarium* root. A linkage map was constructed by placing nine of the polymorphic SSR markers into three partial-linkage groups, each with three SSR markers. The other three markers did not connect to these three linkage groups. Using single marker analysis, two SSR markers that were closely linked to each other (PVBR87 and PVBR109) were significantly associated with *Fusarium* root scores ($p<0.0001$) in K132 x MLB-49-89A population. Another SSR marker, PVBR255, showed significant effects on *Fusarium* root rot scores, but at a reduced significance level ($p\leq0.05$). The other nine SSR markers showed no significant effects. Composite interval mapping detected a major QTL in K132 x MLB-49-89A population between PVBR87 and PVBR109 with a LOD score of 6.1 and coefficient of determination (R^2) of 34% and did not assign independent significance to the distantly-liked marker, PVBR255. Therefore, only one QTL was detected in the present study, but it is a major QTL, as indicated by the large R^2. The two markers associated with the QTL (PVBR87 and PVBR109) are found on linkage group B3 of the common bean core map, close to the region where resistance to root rots, anthracnose, common bacterial blight and bacterial brown spot have been previously mapped. Only four SSR markers (PVBR87, PVBR109, BM156 and BM172) were used in the K20 x MLB 49-89A population because of resource constraints. The two SSR markers (PVBR87 and PVBR109) that were significantly associated with *Fusarium* root rot resistance in K132 x MLB-49-89A population, also showed significant associations (R^2 = 14%, $P < 0.001$) in the K20 x MLB-49-89A population. This is a confirmation of the presence of a QTL identified on linkage group B3 close to these two markers in the K132 x MLB-49-89A population. The association of this major QTL with resistance in both populations suggests that

this QTL may be useful more broadly. There is need to determine whether this QTL is also present in different sources of resistance and whether the two associated SSR markers are useable for marker-assisted selection in a wider range of materials. Fine-resolution mapping could be achieved by using additional markers near the identified markers, enhancing the efficiency of marker-assisted selection and revealing whether this is indeed a single QTL or whether it is made up of several linked QTL, each with a small effect. The detection of this major QTL for resistance to *Fusarium* provides good prospects for using QTL--based approaches to introgress resistance to *Fusarium* root rot from Meso-american genotypes into locally adapted Andean bean genotypes.

DEDICATION

To my beloved family

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TABLE OF CONTENTS

LIST OF TABLES

TABLE PAGE

LIST OF FIGURES

FIGURE PAGE

LIST OF PLATES

PLATE PAGE

CHAPTER ONE

INTRODUCTION

1.1 Importance of Common Bean

The common bean (*Phaseolus vulgaris* L.) is the most important cultivated food legume crop worldwide, accounting for 75% of the food legumes traded in the world (Broughton *et al*., 2003). It is a primary source of protein in the diets of many low-income populations of the world. Beside its high protein content, this bean contains large quantities of complex carbohydrates, fibre, oligosaccharides and phytochemicals, such as polyphenols and isoflavones (Anderson *et al.*, 1999). Common bean is also an important source of iron, phosphorus, magnesium and manganese and also provides lower levels of zinc, iron, copper and calcium (Broughton *et al.*, 2003). The crop is currently the second most important source of human dietary protein and the third most important source of calories for over 100 million people in rural and poor urban communities of Africa (Buruchara, 2006). Beans are grown in a majority of African countries, with about 2.3 million metric tonnes of dry beans produced annually in East Africa alone (FAO, 2009). In some regions of Uganda, beans provide up to 25% of total calories consumed and 45% of total dietary protein. These figures, the highest in the world, are shared by its neighbours Rwanda, Burundi and the Kivu province of the Democratic Republic of Congo (Pachico, 1993). In addition to their nutritional importance, beans have some non-food value too. As a legume, they contribute greatly to soil fertility through symbiotic nitrogen fixation through rhizobia bacteria.

1.2 Constraints to Production of Common Bean

Although common bean is well adapted to various cropping systems and has the advantage of a short growing cycle, it is susceptible to many biotic and abiotic constraints (Wortmann *et al*.,

1998). Important abiotic constraints include low soil fertility, particularly involving deficiency of nitrogen, phosphorus and zinc and toxicities from aluminium and manganese (Singh, 2001). Drought affects bean production most strongly in regions with high temperatures (>30°C in the day time and/or >20°C at night) and at low elevations (below 650 m) in the tropical low lands (Singh, 2001).

Major biotic constraints include pests and diseases. The most important insect pests include leafhoppers [*Empoasca kraemeri* (Ross & Moore)] in the tropics and subtropics and *Empoasca fabae* (Harris) in the temperate and cooler environments (Singh, 2001). Bean fly [*Ophiomyia phaseoli* (Tryon)] is by far the most damaging insect pest of common bean in Africa (Wortmann *et al.,* 1998). The bean weevils [*Zabrotes subfasciatus* (Boheman)] in warm tropical and subtropical environments and *Acanthoscelides obetectus* (Say) in cool and temperate environments cause severe losses when dry beans are not stored properly (Singh, 2001). The levels of significance of the relevant pests depend on climatic conditions and adaptability of the pest.

Singh (2001) lists diseases affecting common bean. He cites bacterial blight as a widespread problem, extending from tropical to temperate environments. In relatively cooler and wetter areas, halo blight [*Pseudomonas syringae* pv. *phaseolicola* (Burkh.)] and bacterial brown spot [*Pseudomonas syringae* pv. *syringae* (van Hall)] may cause severe yield losses. Angular leaf spot [*Phaeoisariopsis griseola* (Sacc.) Ferr.], anthracnose [*Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib.] and rust [*Uromyces appendiculatus* (Pers) Unger] are considered to be the most widely distributed foliar fungal diseases resulting in severe yield losses of common bean in the Americas, Africa and other parts of the world. Web blight [*Thanatephorus cucumeris* (Frank) Donk.] in the warm humid tropics and white mold and ascochyta blight [*Phoma exigua* var. *diversispora* (Bub.) Boerma] in cool wet regions, occasionally become severe on common

bean. Viruses that can cause severe yield losses include bean common mosaic virus (a Potyviridae) in most bean producing regions of the world and bean golden mosaic virus (a geminivirus), which occurs in tropical and subtropical areas (Singh, 2001).

Root rots caused by *Fusarium solani* (Mart.) f.sp. *phaseoli* (Burkholder) N.C. Snyder & H.N. Hans and other soil-borne pathogens that occur in most bean growing environments are a serious problem in common bean production. In Uganda, bean root rot is one of the most serious constraints to production with significant losses occurring among susceptible varieties, especially in the South-Western highlands (Spence, 2002).

Resistance to disease remains an important objective for most bean-breeding programmes. Selecting for greater tolerance to abiotic stresses, such as drought, heat and low soil fertility, is expected to gain importance in response to climate change and increased use of marginal land for bean production. Breeding for these traits is constrained by difficulty to select for novelty. The use of biotechnologies may address this problem. Genome mapping and molecular breeding are tools that bean breeders are increasingly using, especially for improving resistance to diseases and to abiotic stresses (Beaver and Osorno, 2009).

1.3 Justification of the Study

Over the past 12 years there has been a steady increase in the area planted to beans in Uganda, increasing from 615,000 ha in 1996 to 849,000 ha in 2006 (FAO STAT, 2007). However, there has been a general decline in production per unit area. For example, bean production in the country was estimated at 600 Kg/ha in 1999 and 500 Kg/ha in 2006 (FAO STAT, 2007). Decline in production has been attributed to several biotic and abiotic factors, with root rots being a major biotic constraint to bean production in Uganda (Mukankusi, 2007).

Root rots have been reported in most bean fields throughout the world (Park and Tu, 1994). In East Africa and many other parts of Africa, they are responsible for most of the losses in yield (Spence, 2003). In Uganda root rot is one of the major constraints to bean production, especially in the South-Western highlands, with significant losses occurring in susceptible varieties (Tusiime, 2003; Opio *et al*., 2007). Root rots are caused by a complex of soil-borne pathogens such as *Fusarium solani* f.sp. *phaseoli*, *Pythium* spp., *Rhizoctonia solani*, *Macrophomina phaseoli* and *Sclerotium rolfsii* (Rusuka *et al*., 1997). Bean root rot caused by *Fusarium solani* f.sp. *phaseoli* is considered the most serious and wide spread soil–borne disease of common beans, with yield losses of up to 85% attributed to the pathogen (Abawi and Pastor Corrales, 1990; Park and Tu, 1994). *Fusarium* root rot is characterised by reddish-brown lesions along the tap root and hypocotyls. It is particularly severe on large-seeded Andean bean genotypes because of a lack of genetic resistance in these market classes (Abawi and Pastor-Corrales, 1990). The limited genetic variability present in the Andean germplasm, coupled with an emphasis on selection of seed and pod quality traits, appears to have significantly reduced the genetic variability in large-seeded beans (Schneider *et al.,* 2001). Small-seeded genotypes of Meso-american origin, although not completely resistant to root rot, are not as susceptible as the large-seeded types (Abawi and Pastor-Corrales, 1990). They have been used previously as sources of resistance to *Fusarium* root rot (Silbergel, 1987).

The use of resistant varieties is probably the most effective control measure for *Fusarium* root rot, especially for small-scale farmers with limited access to fungicides (Abawi *et al*., 2006). Sources of resistance exist in common beans and have been reported in Africa (Mukankusi, 2007). Most of the resistant genotypes that have been developed and identified are either late maturing, small or black seeded with a climbing growth habit (Beebe *et al.,* 1981). These attributes reduce their acceptability by farmers and they may not be satisfactory parents in breeding programmes for improving resistance to *Fusarium* root rot in the large-seeded Andean

bean varieties (Mukankusi, 2007). None of the commercial bean varieties currently grown in Uganda are resistant *to Fusarium* root rot. The two most popular Andean varieties in Uganda K132 and K20 are susceptible. Large-seeded varieties are the major market class or preferred bean seed types in most parts of Uganda (Mukankusi, 2007). There is therefore a need to improve the resistance of these seed types to *Fusarium* root rot.

Genetic resistance to *Fusarium solani* f.sp. *phaseoli* is polygenically controlled and is strongly influenced by environmental factors (Schneider *et. al.*, 2001). Since traits such as root rot resistance are genetically complex and difficult to evaluate, the efficiency of phenotypic selection is low, resulting in limited progress in breeding (Roman-Aviles and Kelly, 2005). In addition, scoring individual plants can be problematic for genetically complex traits, since an average of several plants or plots for a particular genotype is preferred for traits strongly influenced by environmental factors (Schneider *et. al.,* 2001). Complex inheritance, combined with genetic incompatibility among some genotypes from widely divergent gene pools, have limited the attempts to introgress *Fusarium* root rot resistance into Andean bean cultivars from the Meso-american gene pool (Beebe *et al*., 1981; Román-Avilés and Kelly, 2005).

Increasingly, breeders have been identifying Quantitative Trait Loci (QTL) to enhance efficiency and progress in breeding programmes. This approach overcomes some of the common limitations encountered by conventional selection for quantitative traits (Asins, 2002). Indirect selection for resistance to *Fusarium solani* f.sp. *phaseoli* based on DNA markers linked to the resistance QTL can facilitate improvement of *Fusarium* root rot, as field selection is laborious. Using genetic markers linked to major QTL for *Fusarium solani* f.sp. *phaseoli* resistance may prove valuable. The bean genome has been mapped using various molecular markers. To-date the simple sequence repeat (SSR) genetic markers have been used to saturate genetic maps (Grisi *et al*., 2007). Thus identification of quantitative trait loci could facilitate marker-assisted

selection for *Fusarium* root rot resistance in common bean. The focus of this thesis is to confirm the usefulness of a Meso-american source for transferring *Fusarium* root rot resistance to locally adapted Andean types and to use SSR markers to identify QTL that could assist in transferring that resistance.

1.4 Objectives

1.4.1 Overall Objective

To develop approaches for introgressing resistance to *Fusarium* root rot from Meso-american to locally adapted Andean bean genotypes, based on identifying useable quantitative trait loci (QTL).

1.4.2 Specific Objectives

a) To confirm the usefulness of a Meso-american source (MLB 49-89A) in transferring resistance to *Fusarium solani* f.sp. *phaseoli* into locally adapted Andean types (K20 and K132).

b) To map quantitative trait loci for resistance to *Fusarium* root rot in common beans.

1.4.3 Study Hypotheses

1) Meso-american lines are effective sources of resistance to *Fusarium solani* f.sp. *phaseoli* and can be used to improve the Andean varieties.

2) Some Simple sequence repeat markers co-segregate with QTL for resistance to *Fusarium* root rot and can therefore be mapped and used in marker- assisted selection to improve resistance.

CHAPTER TWO

LITERATURE REVIEW

2.1 Diversity Among Common Bean Cultivars

The genus *Phaseolus* is of American origin and comprises over 30 species (Debouck, 1999). Only five of these species were domesticated, namely *Phaseolus acutifolius* (A. Gray) (tepary bean), *Phaseolus coccineus* L. (scarlet runner bean), *Phaseolus lunatus* L. (lima bean), *Phaseolus polyanthus* Greenman (year-long bean) and *Phaseolus vulgaris* L. (common bean) (Debouck, 1999). Among these species, common bean is the most widely grown, occupying more than 85% of production area sown to all *Phaseolus* species in the world (Singh, 2001). Large variation in growth habit, phenological traits, seed size, shape, colour and canning and cooking qualities are found among dry bean cultivars (Singh, 2001). Genetic diversity in common bean is organised into large-seeded Andean (>40 g 100-seed weight^{-1}) and small-seeded (<25 g 100-seed weight^{-1}) and medium-seeded (25–40 g 100-seed weight^{-1}) Meso-american gene pools (Singh, 2001). Further evidence for the existence of the two gene pools has been demonstrated by the relationship of seed size (small versus large) with: (i) the *Dl* genes (*Dl-1* versus *Dl-2*) and F_1 hybrid incompatibility; (ii) phaseolin seed proteins; (iii) allozymes; (iv) morphological traits; and (v) DNA markers (Singh, 2001). The Andean and Meso-american cultivated gene pools are further divided into six races, each with its own distinguishing characteristics, ecological adaptation and agronomic traits. These include: Andean (all large-seeded) = Chile, Nueva Granada and Peru; Meso-american = Durango (medium-seeded semi-climber); Jalisco (medium-seeded climber); and Meso-american (all small-seeded). The existence of additional diversity within Meso-american races has been reported, especially in a group of Guatemalan climbing bean accessions that do not group with any of the previously defined races, (Beebe *et al.*, 2000).

Growth habit in beans varies from determinate dwarf beans to very vigorous indeterminate climbing beans. Morphologically, beans are classified as determinate or indeterminate, based on whether a terminal reproductive or vegetative meristem is formed at flowering (Voysest and Dessert, 1991). Common bean varieties have evolved during domestication from extremely indeterminate climbing types to determinate bush types, from sensitivity to a long photoperiod to insensitivity, from small- to large-seeded forms, from seed dormancy and water impermeability of the seed coat to lack of dormancy and a water-permeable seed coat and from highly fibrous pod wall and shattering forms to lack of fibers and non-shattering types (Gepts and Debouck, 1991). These varieties are today widely distributed around the world and are cultivated from subsistence to commercial levels.

2.2 Bean Root Rot Diseases

Root rot diseases are widespread and are often considered a major constraint to bean production, reducing both yield and profitability worldwide. Root rots are caused by a complex of fungal pathogens resident in the soil and include *Pythium* spp., *Fusarium solani* f.sp. *phaseoli*, *Rhizoctonia solani* and *Sclerotium rolfsii* (CIAT, 2003). *Fusarium oxysporum* (Schlecht.) f.sp. *phaseoli* is another important pathogen that takes advantage of damage caused by other root rot pathogens to enter the vascular system of the plant, causing *Fusarium* wilt (Rusuku *et al*., 1997).

When evaluated by spatial distribution, plant damage and effect on yield, *Pythium* and *Fusarium* species are especially important,particularly under conditions of high soil moisture/rainfall and low temperatures that favour disease development (CIAT, 2003). Over the last decade, the incidence and severity of bean root rots have increased markedly in the Great Lakes Region of Central Africa and East Africa, resulting in a general decline in bean production (CIAT, 2003). Moreover, bean root rots are among diseases and pests associated with intensification of agriculture (CIAT, 2003).

Symptoms of root rots induced by *Fusarium solani* f.sp. *phaseoli* appear soon after the seedling emerges. The tap root is slightly discoloured at first, gradually becoming brick red turning to brown, with longitudinal cracks. The fibrous roots at the bottom are usually killed and new fibrous roots may form just above the discoloured area and just below the soil surface. Continuous bean cropping allows a build-up of the fungus in the soil. The fungus can survive for 5 years or longer in the absence of the crop (McNab, 2007).

The *Pythium* spp. (*Oomycete*) that cause *Pythium* root rot are known to survive in the soil for several years as oospores that infect the root and lower stem (Rusuku *et al.*, 1997). Depending on the time of infection and environmental conditions, disease symptoms caused by *Pythium* spp. may appear as seed rot (before germination), damping-off, root rot, foliar blight or pod rot (Abawi and Pastor-Corrales, 1990). Initial infection symptoms appear as elongated, dark-brown, water-soaked areas on root and lower stem tissues. Infected tissues become brownish, soft, sunken and eventually collapse, causing plant wilt and death (Otsyula *et al.*, 2003).

McNab (2007) points out that *Rhizonctonia* root rot, caused by *Rhizoctonia solani*, is characterised by seed rot and a water-soaked stem rot near the soil line, which in new seedlings often results in wilt and death. More commonly, slightly sunken reddish-brown longitudinal stem cankers appear near the soil line on older plants. As inner stem tissue is invaded, it becomes brick red.

The involvement of multiple soil-borne pathogens that have different mechanisms of pathogenicity has made it difficult to develop a simple and effective disease management program. Currently, an integrated approach to disease management, using a combination of compatible, appropriate and complementary methods, is considered an effective strategy (CIAT, 2003). The use of resistant, adapted and acceptable cultivars is an effective management option

for disease control, particularly for small-scale farmers (CIAT, 2003). In the identification of genetic options, evaluation of over 4000 diverse germplasm accessions has resulted in only a few resistant entries (less than 2.5 %), which emphasizes the limitation of relying on resistant varieties in managing such a complex problem (CIAT, 2003). The focus of this thesis is on *Fusarium solani* f.sp. *phaseoli*, one of the major pathogens that so far has received limited efforts toward genetic improvement of resistance.

2.3 *Fusarium* Root Rot

2.3.1 The Pathogen and Disease Symptoms

Fusarium root rot is caused by the fungus *Fusarium solani* f.sp. *phaseoli* and belongs to the Nectria haematococca-*Fusarium solani* species complex, section Martiella, of *Fusarium* (O'Donnel, 2000). The fungus is homothallic, although some strains are heterothallic. It is one of the ten *formae specialles* of *Fusarium solani* [Teleomorph Haematonectria haematococca Syn. Nectria haematococca] (Rossman *et al.,* 1999).

The pathogen kills the tissue around the basal part of the stem and root system and the characteristic symptom of damage on beans is the reddish discoloration of the tap root and the desiccated lower stem (Erwin *et al.,* 1991). The first symptoms are narrow, long, red to brown streaks on the hypocotyls and tap root. The tap root later turns dark brown and cracks often develop lengthwise. It may then shrivel and die, with clusters of fibrous roots developing above the shrivelled tap root. These fibrous roots may keep the plant alive and under ideal conditions, a few above-ground symptoms appear. Plants may be stunted, have abnormal colour and grow more slowly than healthy plants, resulting in an uneven plant stand (Abawi *et al*., 2006). *Fusarium* root rot is favoured by temperatures of 14-24^{o}C, although the optimum temperature is around 21^{o}C (Sippel and Hall, 1982).

2.3.2 *Fusarium* Root Rot Etiology and Epidemiology

The pathogen usually survives as thick-walled chlamydospores in the soil (Sikora, 2004). These spores germinate when stimulated by nutrients exuded by germinating seeds and root tips. Then the fungus penetrates the plant tissue. Chlamydospores of *Fusarium* can germinate and reproduce near the seed and roots of many non-host plants as well as organic matter (Sikora, 2004). This means that the pathogen can survive in the field indefinitely. The pathogen is then dispersed by wind, rain, irrigation water, farm implements and any other agent or process capable of moving soil. With each successive crop of beans, pathogen populations increase and the disease becomes more severe. Plant damage is usually increased under environmental conditions that stress plants. These conditions include deep planting, soil compaction, hardpan layers, cool temperatures, high or low pH, low fertility, pesticide or fertilizer injury and flooding or extended drought (Sikora, 2004).

In soil, the pathogen spores are often under the influence of soil fungistasis (Hall, 1991). However, when fungistasis is reversed, they germinate and penetrate bean tissue directly or through wounds and natural openings (Hall, 1991). Soil fungistasis is reversed when spores are stimulated by nutrients exuded by germinating been seeds and root tips. The fungus then grows intercellularly throughout the cortical tissues (Kraft *et al.,* 1981).

2.3.4 Mechanisms of Resistance to *Fusarium solani* f.sp. *phaseoli*

The underlying basis of resistance is varied. Mechanisms associated with host defence response are involved in resistance. For example, a hypersensitive reaction to infection by *Fusarium solani* f.sp. *phaseoli* has been reported (Pierre and Wilkinson, 1970). Another mechanism of resistance to *Fusarium* root rot is possession of vigorous root system (Snapp *et al.,* 2003). Of particular interest is the partitioning of carbohydrate between shoots and roots. Strong root

systems are associated with better partitioning and therefore better resistance to root rot (Snapp *et al.,* 2003).

The colour of seed and hypocotyls has also been related to the level of resistance to *Fusarium solani* f.sp. *phaseoli.* Small and black-seeded varieties are reported to be more resistant compared to large red mottled ones (Beebe *et al.,* 1981). Statler (1970) observed higher resistance to *Fusarium solani* f.sp. *phaseoli* in black- seeded varieties and varieties with purple-coloured hypocotyls and related it to the higher production of phenolic compounds inhibitory to fungal growth in the early stages of seedling growth. Phytoalexins such as phaseollin produced in response to infection by *Rhizoctonia solani* and *Fusarium solani* f.sp. *phaseoli* may also enhance resistance to pathogen attack (Kendra and Hadwiger, 1984). Production of these phytoallexins has been shown to be higher and more rapid in resistant bean varieties than in susceptible ones.

2.3.5 Heritability of Resistance to *Fusarium* Root Rot

Various studies have demonstrated that resistance in common bean to *Fusarium* root rot is heritable (Schneider *et al*., 2001; Roman-Aviles and Kelly, 2005). Moderate to high heritability estimates have been reported. The heritability estimates vary depending on whether the data is based on the field estimates or green house. Generally, estimates from green house data tend to higher because of the reduction of the variability attributed to the environment. Hassan *et al*., (1971) reported broad sense heritability of resistance to *Fusarium* root rot ranging from 0.61 to 0.64 under greenhouse conditions and 0.77 to 0.79 under field conditions. The narrow sense heritability varied from 25.9% to 44.3% for the inter-genepool crosses (Hassan *et al*., 1971). In previous studies within a single gene pool, higher narrow sense heritabilities have been found, ranging from 0.48 to 0.71 in F_4-derived recombinant inbred lines (Schneider *et al*., 2001). Narrow sense heritability for estimates for *Fusarium* root rot resistance ranged from 0.1 to 0.51

for the kidney and from 0.2 to 0.82 for the cranberry inbred backcross populations (Román-Avilés and Kelly, 2005). Broad sense heritability estimates ranged from 0.22 to 0.69, with a narrow sense heritability of 0.34 in populations involving crosses between Meso-american and Andean lines (including K20, K132, MLB-49-89) (Mukankusi, 2007). The high to moderate heritability previously reported indicate that once suitable sources of resistance have been identified, introgression of resistance from resistant to susceptible genotypes should be possible.

2.3.6 Sources of Resistance to *Fusarium* Root Rot

Common bean varieties with resistance to single or multiple root rot pathogens have been reported in Africa (Mukankusi, 2007). However, none of the commercial bean varieties currently grown in Uganda exhibit a high level of tolerance to pathogens that cause *Fusarium* root rot. Small and black-seeded Meso-american varieties are generally more resistant to *Fusarium solani* f.sp. *phaseoli* than are the large and red seeded varieties (Beebe *et al.,* 1981). Most of the resistant genotypes already available are late maturing, small or black seeded with a climbing growth habit (Beebe *et al.,* 1981). A resistant large-seeded cultivar, FR266 that belongs to the Andean genepool has been developed using a small and black seeded variety (N203, a Meso-american genotype) as source of resistance (Silbernagel, 1987). Schneider *et al.* (2001) successfully used FR266 as a source of resistance to *Fusarium* root rot in crosses with beans from the Andean gene pool. The major challenge is that some of these varieties that are resistant to *Fusarium* root rot are adapted to the USA and Latin America but not to African environments that probably have different pathogens and pathogen strains (Mukankusi, 2007). The small-seeded genotypes of Meso-american origin, although not completely resistant to root rot, are valuable sources of resistance (Abawi and Pastor-Corrales, 1990). Bean varieties that are moderately resistant to *Fusarium* root rot and grown by farmers in Kenya, Rwanda and Uganda include MLB-49-89A, RWR 719, SCAM-80-CM/15, MLB-49-89A and RWR 1092 (CIAT, 2003). Meso-american varieties MLB-49-89A and RWR 719 have been widely adopted and have

had a major impact by reducing root rot epidemics in Western Kenya where bean production had virtually stopped due to root rots (CIAT, 2003). It is therefore important to study the effectiveness of using Meso-american genotypes that are adapted to African climate with adequate levels of resistance to *Fusarium* root rot as sources of resistance to improve the susceptible Andean varieties. Over emphasis on improvement of quality traits has led to neglect in improvement of disease resistance in kidney and snap beans (Schneider *et al*., 2001). This may be responsible for the high susceptibility to *Fusarium solani* f.sp. *phaseoli* in these seed types as compared to the small-seeded beans (Roman-Aviles and Kelly, 2005).

2.3.7 Challenges of Breeding for Resistance to *Fusarium* Root Rot

Breeding for resistance to *Fusarium* root rot is difficult because environmental conditions and soil types contribute to increased disease severity in regions where large-seeded beans are produced (O'Brien *et al.,* 1991; Estevez de Jensen *et al.,* 1998). Lack of field uniformity for inoculum and disease pressure can also compound the breeding progress (Boomstra and Bliss, 1975). Large experimental errors due to field heterogeneity and large genotype x environment interactions have also contributed to the lack of progress in breeding for field resistance to the root-rot complex (Boomstra and Bliss, 1975). Breeding for root rot resistance in beans requires a homogeneous experimental site with high disease potential and an appropriate experimental design to permit discrimination among genotypes (Navarro *et al.,* 2008).Improvement of resistance to *Fusarium solani* f.sp. *phaseoli*, especially in large-seeded dry and snap bean types, has been limited, in spite of considerable research efforts to elucidate its genetic control. *Fusarium* root rot is particularly severe on large-seeded Andean bean genotypes due to lack of genetic resistance (Abawi and Pastor-Corrales, 1990; Schneider *et al.*, 2001). In addition, genetic diversity in the cultivated Andean genotypes is generally very limited (Islam *et al.*, 2004). Development of tools and of approaches for breeding of resistance requires a clear understanding of the nature of resistance as well as the tools to support breeding. The use of genome mapping

and molecular breeding are additional tools that bean breeders are increasingly using to more effectively breed for resistance to diseases and abiotic stresses (Beaver and Osorno, 2009).

2.4 Advances in Generating the Bean Genetic Map

The *Phaseolus vulgaris* L. genome has an estimated size of 650 million base pairs (Mbp) distributed among 11 chromosomes (Arumuganatham and Earle, 1991). Genetic maps for common bean have been available since the 1990s and are based on various types of molecular markers. The major genetic markers include the co-dominant Restriction Fragment Length Polymorphism (RFLP) markers and dominant Random Amplified Polymorphic DNA (RAPD) markers (Vallejos *et al*., 1992; Nodari *et al.*, 1993; Adam-Blondon *et al.*, 1994; Vallejos, 1994). A consensus genetic map for common bean that covers a genetic distance of 1226 cM has been developed by integrating marker information from different populations into a reference map derived from the segregation of 563 markers in a common population (BAT93 X Jalo EEP558) (Freyre *et al*., 1998). An increasing number of genetic maps are being developed in common bean for the identification of quantitative trait loci and of identified genes that control important traits of economic interest. Nevertheless, the information tends to be restricted to those populations already mapped, since the transferability of the molecular markers is very low. Poor transferability is a constraint for comparative genome studies and consequently, for information exchange across different maps (Grisi *et al.*, 2007), indicating the need more mapping efforts especially in local or target populations. Use genetic markers such as SSR markers may help to solve the challenge of transferability of marker information between genotypes and genetic maps.

2.4.1 SSR Markers and Mapping Efforts

SSR markers are useful for genetic studies because they are co-dominant, multi-allelic, widely distributed across the genome, polymerase chain reaction (PCR)-based and transferable between

different genotypes (Grisi *et al*., 2007). Information generated by these markers allows for comparison and exchange of information between different studies, especially in comparative genetic mapping (Grattapaglia, 2000). Recently, several research groups have made advances in the development of SSR markers for various species of the Leguminosae (Song *et al*., 2004; Wang *et al*., 2004). For common bean, SSR markers have been developed from gene bank sequences and enriched genomic libraries (Grisi *et al*., 2007). The first GenBank derived microsatellites were a set of 38 (Yu *et al*., 1999, 2000). Subsequently, Blair *et al.* (2003) and Guerra-Sanz (2004) developed 57 and 20 additional SSR markers, respectively. Yaish and Perez De La Vega (2003) isolated an additional series of 21 SSR markers. Caixeta *et al.* (2005) used bacterial artificial chromosome libraries to develop SSR markers linked to a resistance gene. To date, a genetic map has been constructed exclusively with SSR makers, with 106 SSR markers placed in 12 groups with a total length of 606.8 cM and average distance of 6.8 cM (Grisi *et al*., 2007). Thus, adequate numbers of SSR markers have been mapped in the common bean genome that can facilitate identification and mapping of QTL for several traits of economic importance.

2.5 Mapping of QTL for *Fusarium* Root Rot Resistance: Past Experiences

Analyses of recombinant inbred lines and other mapping populations have led to the identification of QTL contributing to *Fusarium* root rot resistance in common bean (Schneider *at el.*, 2001; Chowdhury *et al.*, 2002; Román-Avilés and Kelly, 2005). However, no SSR markers were used in these studies. Because of the many advantages of using SSR markers, the focus of this study is to use SSRs to identify and localise the QTL to *Fusarium* root rot, building on several previous mapping studies of *Fusarium* root rot in common bean.

Sixteen QTL for *Fusarium* resistance were identified using $F_{4:5}$ recombinant inbred lines derived from a cross between the susceptible large-seeded red kidney ‘Montcalm’ and the root-rot-resistant snap bean breeding line FR 266 (Schneider *et al.*, 2001). Individual RAPD markers in

that study each explained more than 15% of the observed phenotypic variation for *Fusarium* root rot resistance (Schneider *et al.,* 2001). Two of the markers that showed a significant association with resistance are located on linkage group B2 of the bean consensus genetic map and span a region that encompasses the *PvPR2* locus. This suggests a role of this pathogenesis-related protein in root rot resistance (Schneider *et al.,* 2001). *PvPR 2* and its counterpart *PvPR1* are acidic proteins of low molecular weight that are induced during fungal infection (Walter *et al.,* 1990).

Interval mapping has revealed two QTL for resistance to *Fusarium* root rot using an $F_{2:6}$ recombinant inbred line population (Chowdhury *et al.,* 2002). In that study, one QTL was located between UBC218 1200 and UBC 503640 and the other was located between UBC 503 640 and UBC 211 100. The first QTL had a Likelihood of Odds (LOD) score of 8.0 and explained 30% of the phenotypic variation. The other QTL had an LOD score of 5.0 and explained about 20% of phenotypic variation (Chowdhury *et al.,* 2002).

Six QTL for resistance to *Fusarium* root rot have been identified using a recombinant inbred lines derived from a cross between the root-rot-susceptible snap bean 'Eagle' and '*Puebla* 152', a small black-seeded root-rot-resistant dry bean (Navarro *et al*., 2004). Most of these QTL are located on linkage groups B2 and B3 of the integrated bean map, close to the location of response genes polygalacturonase-inhibiting protein (*PGIP*) and chalcone synthase locus (*ChS)* and the defence and pathogenesis-related proteins, *PvPR-1* and *PvPR-2* (Freyre *et al*., 1998; Schneider *et al.,* 2001). The co-localisation with genes of known function suggest a possible mechanisms of QTL in *Fusarium* root rot, while the genetic diversity among resistance sources emphasises the need for cyclic breeding systems to combine QTL located in diverse genomic regions (Román-Avilés and Kelly, 2005).

Nine QTL that showed significant associated with *Fusarium* root rot resistance in the field and green house have been identified using Random Amplified Polymorphic DNA markers in two inbred backcross-derived populations (Román-Avilés and Kelly, 2005). In that study, QTL associated with *Fusarium* root rot resistance were identified on linkage groups 1, 5, 7, 8 and 9. Three linkage groups (1, 7 and 9) possessing QTL associated with root rot resistance co-segregated with linkage groups B2 and B5 of the integrated map (Román-Avilés and Kelly, 2005).

Other resistance factors previously mapped to B5 include QTL for resistance to common bacterial and halo blight (*Pseudomonas syringae* pv. *phaseolicola*) and the lipoxygenase gene, *Lox-1*, required during development of resistance in beans plants under desiccation stress (Porta *et al.*, 1999). The QTL of major effect that was detected on B2 is in the vicinity of the *ChS*, *PGIP* and the pathogenesis-related protein, *PvPR-2* (Román-Avilés and Kelly, 2005). Plant defence response is a complex mechanism that is triggered by pathogen attack. In beans, several defence-response genes co-localize with resistance QTL suggesting a functional relationship (Geffroy *et al.*, 2000). Other QTL for resistance to root rot and white mold have been previously mapped to regions close to *ChS, PGIP* and the *PVPR-2* on B2, suggesting that physiological resistance to *Fusarium* root rot and white mold [*Sclerotinia sclerotiorum* (Lib.) de Bary] is associated with a generalised host defence response (Schneider *et al.*, 2001; Kelly and Vallejo, 2005).

Recombinant inbred lines from 'Eagle' x 'Puebla' and two inbred backcross populations were evaluated for root rot resistance by plant stand, plant vigour and plant biomass (Navarro *et al* 2008). Using composite interval mapping with a LOD score threshold of 2.0, five regions from linkage groups B6, B3 and B7 of the *Phaseolus vulgaris* core map were associated with root rot resistance (Navarro *et al.*, 2008).

2.6 QTL Mapping of Other Disease Resistance Loci in Common Bean

2.6.1 Anthracnose

Over 10 major genes conditioning resistance to anthracnose have been identified, with markers showing linkage to six independent dominant genes (*Co-1, Co-2, Co-4*^{2}*, Co-5, Co-6, Co-9*) (Geffroy *et al.*, 1999; Kelly *et al*., 2003). In addition to genetic studies that show resistance to anthracnose resides at multi-allelic loci, mapping studies have confirmed that these loci reside on various linkage groups of the core bean map (Freyre *et al.,* 1998; Meloto and Kelly, 2000). The *Co-1* locus is located on linkage group B1; *Co-2* on B11; *Co-4* on B8; *Co-6* on B7 and *Co-9* on B4 (Gefffroy *et al.,* 1999; Miklas *et al*., 2000).

2.6.2 Bean Common Mosaic Virus and Bean Common Mosaic Necrosis Virus

Resistance to bean common mosaic virus in common bean is conditioned by a series of multi-allelic loci (Drijfhout, 1978). The dominant *I* gene, located on linkage group B2 core bean map, is independent of three recessive *bc* loci (Gepts, 1999). The *bc-3* gene is located on linkage group B6 whereas the *bc-1*^{2} allele resides on linkage group B3 (Miklas *et al.*, 2000). The pathotype non-specific allele, *bc-u,* also resides on linkage group B3 based on loose linkage with the *bc-1* locus (Strausbaugh *et al.*, 1999).

2.6.3 Bean Golden Mosaic Virus

Resistance to bean golden mosaic virus is conditioned by the recessive gene *bgm-1* and *bgm-1* (Urrea *et al.*, 1996). A co-dominant RAPD marker tightly linked with *bgm-1* has been identified and is being used by breeders to hasten the development of bean germplasm with moderate resistance levels (Urrea *et al.*, 1996). In addition to the resistance conditioned by major genes, two independent QTL were found that have a major effect on reduced mosaic resistance in the cultivar Dorado (Miklas *et al.,* 1996). Together these QTL explained 60% of phenotypic

variation in disease reaction in one environment and were consistently expressed across three separate field environments. One of the QTL is located on linkage group B4 based on a Sequence Characterised Amplified Region developed from the RAPD OWR700 (Miklas *et al.*, 2000). The QTL on linkage group B4 is also negatively associated with resistance to *Macrophomina phaseoline* present in the second parent (XAN176) of the mapping population (Miklas *et al.*, 2000). The second resistance QTL resides on linkage group B7, near the *Asp* and *Phs* loci, where the study also found QTL conditioning resistance to common bacterial blight, white mold (causal organism *Sclerotinia Sclerotiorum*), anthracnose and *Macrophomina* (Geffroy, 1997).

2.6.4 Bacterial Diseases

Five QTL that confer resistance to *Xanthomonas axonopodis* pv. *phaseoli* have been identified, with one QTL located on each of linkage groups B2, B5, B7 and B9 (Nodari *et al.*, 1993b). In other studies, seven QTL conditioning resistance to bacterial blight (*Xanthomonas campestris*) have been reported in common beans (Young, 1996). These seven QTL jointly explain 75% of the variation in resistance to bacterial blight, with each locus explaining 11-35% of the variation (Young, 1996).

2.7 Resistance Gene Clusters in Common Bean

Many resistance genes occur in clusters that protect the plant against several pathogens (Michelmore and Meyers, 1998). In common beans, genes for disease resistance are also concentrated in different regions of the common bean genome (Kelly *et al.*, 2003; Miklas *et al.*, 2006). Therefore, there is need for bean breeders to understand the genetic variation of these genes. QTL analysis is a suitable tool for identifying gene clusters and tagging QTL presents an opportunity for effective introgression of disease resistance. In common beans, genes for resistance to anthracnose (*Co-3/Co-9*), rust (*Ur-5*) are linked with the SW-12 QTL for resistance

to Bean Golden-yellow mosaic virus (BGYM) (Mendez-Vigo *et al*., 2005). The anthracnose resistance genes *Co-3/Co-9* and *Co-2* are organised in two clusters, suggesting that genes for anthracnose resistance could be organised into clusters that confer race-specific resistance (Rodriguez-Suarez *et al*., 2007).

2.8 Approaches and Methods for QTL Mapping

A Quantitative Trait Locus (QTL) is a chromosomal region that is likely to contain causal genetic factors for the phenotypic variation under study (Zou and Zeng, 2008). QTL mapping is based on the basic principle that if there is linkage disequilibrium between the causal factor and a marker locus, mean values of the trait under study will differ among genotype groups with different genotypes at the marker locus (Zou and Zeng, 2008). QTL mapping therefore involves the following steps: (i) Constructing a mapping population from two parents; (ii) identifying candidate markers and screening them for polymorphism; (iii) constructing a linkage map; (iv) analyzing for QTL-trait association using single-marker analysis, interval mapping, composite interval mapping or any other method.

2.8.1 Population Development

A mapping population is one that segregates for the trait of interest. Subsequently, the population is genotyped for segregating markers targeted to specific chromosome regions and/or markers evenly distributed over a genome-wide genetic map. The segregating genotypes characterised phenotypically for quantitative and/or qualitative traits of (Alonso-Blanco *et al.,* 2006). Mapping populations are based on segregating progeny, often derived from F_1 hybrids between parents that strongly contrast for the trait of interest (Alonso-Blanco *et al.,* 2006). Such populations have pronounced strong linkage disequilibrium between loci, allowing the detection of linkage between markers and the trait of interest. Specific genotypes from the mapping population may

be intercrossed with each other or outcrossed to other specific genotypes in order to detect only markers closely linked to the QTL.

2.8.1.1 Balanced Populations

Different populations may be used for mapping within a given plant species, each conferring its own advantages and disadvantages. F_2 populations, derived from F_1 and backcross populations (derived from F_1 or F_2 plants crossed to one or both parents) are the simplest types of mapping populations developed for self pollinating species (Collard *et al.,* 2005). Their main advantages are that they are easy to construct and require only a short time to produce. Inbreeding from individual F_2 plants forms recombinant inbred lines, which consist of a series of nearly homozygous lines each containing a unique combination of chromosomal segments from the original parent. Balanced populations such as recombinant inbred lines, F_2 and doubled haploid populations in which both parental alleles are present in almost equally high frequencies have been used most frequently in QTL studies (Butruille *et al.,* 1999). The estimation of the number of QTL and of the relative position and contribution of each QTL to the expression of a trait of interest is determined most efficiently in balanced populations.

Evaluation of highly homozygous families, such as recombinant inbred lines, offers several advantages when compared to the evaluation of F_2 plants or F_3 families. The advantages include: (1) Very limited heterozygosity, which in turn allows for the more effective use of dominant markers; (2) Greater genetic variability among families due to stronger expression of additive effects; (3) Higher mapping resolution due to the higher number of crossover events and (4) the opportunity to more consistently reproduce the phenotypic evaluations across space and time (Tuberosa *et al.,* 2003). Recombinant inbred lines have been widely used in beans to map the QTL's associated with resistance to a wide range of diseases (Román-Avilés and Kelly, 2005). The population size required for QTL mapping depends on the population type and the degree of

precision of phenotypic evaluation. Evaluation of multi-plant families (F_3 or beyond) provides better phenotypic data than evaluation of single F_2 plants and genetic differentiation is greater among recombinant inbred lines than among F_2 plants. Therefore, recombinant inbred populations have a greater power to detect associations between markers and QTL than F_2 populations representing the same number of genotypes.

2.8.1.2 Unbalanced Populations

An alternative to using a balanced population for QTL mapping is using advanced generations of a backcross population. In such a population, the alleles of one parent are present at a much lower frequency (Tanksley and Nelson, 1996). Unbalanced populations have been used in QTL mapping to determine the number of genes controlling a quantitative trait and to introgress desirable QTL from unadapted to better adapted germplasm, (Tanksley and Nelson, 1996; Doganlar *et al.,* 2002). When using unbalanced populations for mapping and identifying QTL, there is a loss of resolution and efficiency due to the unequal allele frequency inherited in inbred lines from backcross populations (Tanksley and Nelson, 1996; Butruille *et al.*, 1999). However, inbred backcross lines still contribute linkage information to genetic maps (Doganlar *et al.*, 2002) and unbalanced populations have the advantage of being more genetically and phenotypically similar to the recurrent parent. The advantage of using such populations is the recovery of genetic materials that possess the advantages of the recurrent parent but with the addition of desirable alleles from the donor parent.

2.8.2 Identification of Polymorphism

Another step in QTL mapping is to identify DNA markers that reveal differences between parents (i.e. polymorphic markers). It is critical that sufficient polymorphism exist between parents in order to construct a linkage map (Young, 1994). In most cases parents that provide adequate polymorphism are selected on the basis of their level of genetic diversity (Collard *et al*.,

2005). In common beans, crosses involving parents with diverse genetic backgrounds are desirable for genetic mapping. Such crosses have a higher number of segregating loci, since such parents have an increased level of polymorphism, as a result of being from separate gene pools, derived from wild beans that diverged before domestication (Grisi *et al*., 2007).

2.8.3 Linkage Analysis and Construction of Linkage Maps

After identifying polymorphic markers, a linkage map is constructed by recording genotype data for each DNA marker on each individual of a mapping population and then using computer programmes to analyze for linkage between markers and phenotypic traits. The likelihood that particular markers are linked is usually expressed using the odds ratio, i.e., the ratio of the probability of linkage versus the probability of no linkage (Collard *et al*., 2005), expressed as the logarithm of the ratio and called a Logarithm Of Odds (LOD) (Collard *et al*., 2005). A LOD score value of 3 between two markers indicates that linkage is 1000 times more likely (1000:1) than no linkage. Lower LOD values may be used in order to detect more distant linkage and to place additional markers within maps that have been constructed using higher LOD values (Collard *et al*., 2005)..

2.8.4 Methods of Detecting Quantitative Trait Loci

2.8.4.1 Single-Marker Analysis

Single-marker analysis is used for detecting QTL associated with single markers and does not require a linkage map. It is based on the principle of detecting an association between phenotypic expression and the genotype of the DNA markers. DNA markers are used to partition the mapping population into different genotypic groups in order to determine whether significant differences exist between groups with respect to the trait being measured (Collard, *et al.,* 2005). A significant difference between phenotypic means of the groups indicates that the marker locus

is linked to a QTL controlling the trait. Three essentially equivalent statistical methods are used for single marker-analysis: t-test, analysis of variance and linear regression (Collard, *et al.*, 2005). Single–marker analysis simply detects a linkage association of the trait with an individual marker, but does not indicate how close the QTL is to the marker (Collard, *et al.*, 2005). When investigations focus on questions of genomic location, then more robust methods of QTL analysis such as interval mapping and composite interval mapping are used and these rely on the estimated order of the markers (linkage map). The contribution to the total phenotypic variance of the genetic effect attributed to a single locus (indicated as R^2) is estimated through standard regression approach (Tuberosa *et al.*, 2003). The main limitation of single marker analysis is that the effect of any detected QTL will normally be underestimated due to recombinations between the marker locus and the QTL (Tanksley, 1993).

2.8.4.2 Interval Mapping

Interval mapping uses an estimated genetic map as the framework to discover the location of the QTL (Collard, *et al.*, 2005). The intervals, defined by ordered pairs of markers, are searched in increments (for example, 2cM) and statistical methods are used to test whether a QTL is likely to be present within that interval. This likelihood is expressed as aLOD score, computed as the base-10 logarithm of the ratio between the chances of a real QTL being present given the phenotypic effect associated with that position compared to the chance of having a similar effect with no QTL being present (Tuberosa *et al.*, 2003). The peak of the LOD profile indicates the most likely position of the QTL.

2.8.4.3 Composite Interval Mapping

More recently, Composite interval mapping has become popular for mapping QTL. Composite interval mapping is based on a joint regression involving a possible QTL within an interval and

marker loci outside that interval (Doerge *et al*., 1997). It combines interval mapping with linear regression by evaluating a statistical model that includes both the adjacent pair of linked markers being evaluated for interval mapping and one or more additional genetic markers at other chromosomal positions (Jansen and Stam, 1994). The main advantage of composite interval mapping is that it is more precise and effective at mapping QTL than is single-marker analysis or interval mapping, especially when linked QTL are involved (Collard *et al.,* 2005). Most of the current methods of QTL analysis, in terms of experimental design, population dimension and statistical approach are inadequate for detecting epistatic QTL's, which may represent a strong source of variation for complex traits (Tuberosa *et al.,* 2003). In this thesis I use the methods described above in an attempt to detect QTL associated with resistance to *Fusarium* root rot.

2.9 Sectional Conclusion

Genetic resistance to *Fusarium solani* f.sp. *phaseoli* is polygenic and is strongly influenced by environmental factors that confound the expression and detection of resistance mechanisms. Common bean varieties with resistance to root rot pathogens have been reported in Africa. However, none of the commercial bean varieties currently grown in Uganda exhibit resistance to pathogens that cause *Fusarium* root rot. Small and black-seeded Meso-american varieties are in general more resistant to *Fusarium* root rot than are the large and red seeded varieties. Most of the resistant genotypes that have been developed or identified are either late maturing, small or black seeded, with a climbing growth habit. The challenge is that some of these varieties that are resistant to *Fusarium* root rot are adapted to the USA and Latin America climates and so may not be effective sources of resistance in tropical African environments that likely involve different pathogens and pathogenic strains. It is therefore important that we investigate the effectiveness of improving Andean varieties that are susceptible to *Fusarium* root rot by using Meso-american

lines that are already adapted to African climates and possess adequate levels of resistance to this disease.

Genetic maps for common bean have been available since the 1990s and are based mainly on RFLP and RAPD markers. Recently a genetic map has been constructed exclusively with SSR makers. Quantitative trait loci for *Fusarium* root rot resistance have been previously mapped using RAPD markers. There is no published work yet to map quantitative trait loci for *Fusarium* root rot resistance using SSR markers. Such work would be useful in identifying significant QTL-SSR marker association that can be used in marker-assisted breeding. The focus of this thesis is to confirm the usefulness of a Meso-american source for transferring *Fusarium* root rot resistance to locally adapted Andean types and to use SSR markers to identify QTL that could assist in transferring that resistance.

CHAPTER THREE

USEFULNESS OF A MESO-AMERICAN SOURCE (MLB 49-89A) IN TRANSFERRING RESISTANCE TO *FUSARIUM* ROOT ROT INTO LOCALLY ADAPTED ANDEAN GENOTYPES (K20 AND K132)

3.1 Introduction

The use of resistant varieties is probably the most effective control measure for *Fusarium* root rot, especially for small-scale farmers with limited access to fungicides (Abawi *et al*., 2006). Sources of resistance to *Fusarium solani* f.sp. *phaseoli* exist in common beans and have been reported in Africa (Mukankusi, 2007). However, none of the commercial bean varieties currently grown in Uganda are resistant to this pathogen. Most of the developed and identified genotypes are either late maturing, small or black-seeded with a climbing growth habit (Beebe *et al.,* 1981). These attributes reduce their acceptability to farmers and thus they may not be satisfactory parents in breeding programmes for improving resistance to *Fusarium* root rot in large-seeded Andean bean varieties that are popular in Uganda (Mukankusi, 2007).

Fusarium root rot is particularly severe on large-seeded Andean bean genotypes due to lack of genetic resistance in them (Schneider *et al.,* 2001). Although small- and black-seeded genotypes of Meso-american origin are not completely resistant to *Fusarium solani* f.sp. *phaseoli*, they are more valuable sources of resistance than the large- and red-seeded varieties (Beebe *et al.* 1981; Abawi and Pastor-Corrales, 1990). The large-seeded Andean genotypes such as K20 and K132 are the preferred market class of common beans widely grown in Uganda despite being highly susceptible to *Fusarium* root rot. Resistance to *Fusarium* root rot has been identified in some Meso-american lines, such as MLB-49-89A. This study was undertaken to confirm the usefulness of a Meso-american source (MLB 49-89A) in transferring resistance to *Fusarium solani* f.sp. *phaseoli* into locally adapted Andean genotypes (K20 and K132).

3.2 MATERIALS AND METHODS

3.2.1 Description of Study Site

This study was carried out in Uganda at the National Agricultural Research Laboratories (NARL) located 13 km north of Kampala, where the bean research facilities are found. It is at an elevation of 1300 m above sea level. The development of the two populations used in this study was done in the screenhouse. The preparation of inoculum was done at the National Biotechnological centre at NARL. Inoculation and disease evaluation were done in a screenhouse specifically designed for root rot evaluation in common beans. The diurnal temperature in the screenhouse was 27.3^{o}C (maximum) and 15^{o}C (minimum), with a relative humidity of 76.3%.

3.2.2 Germplasm and Bean Populations used in the Study

Crosses of K20 and K132 with MLB-49-89A were used in this study, along with the three parents. Both K20 and K132 are susceptible to *Fusarium* root rot caused by *Fusarium solani* f.sp. *phaseoli* (Mukankusi, 2007). Variety K20 was developed and in Uganda and is widely grown in the country. It belongs to the Andean gene pool, is large-seeded (37.3 g per 100 seeds) and exhibits type I upright determinate bush growth habit (Nabukalu, 2008). It has a red mottled seed colour and takes approximately 33 days to flower. Variety K132 was developed in Uganda and is currently the most widely grown genotype in the country. It also belongs to the Andean gene pool, is large-seeded (44.6 g per 100 seeds) and exhibits the Type I upright determinate bush growth habit. It has a red mottled seed colour and takes approximately 35 days to flower (Nabukalu, 2008). Variety MLB-49-89A is a Meso-american genotype that is moderately resistant to *Fusarium* root rot with black, small to medium seed size (Mukankusi, 2007).

Two populations of recombinant inbred lines were used for this study. A total of 90 and 78 $F_{4:5}$ recombinant inbred lines were derived from the crosses K20 x MLB-49-89A and K132 x MLB-49-89A, respectively. Both populations were developed by advancing F_2 progeny to the F_4 generation through single seed descent. Individual F_4 plants were harvested and the seed from each plant bulked to constitute the F_4 derived recombinant inbred lines ($F_{4:5}$). No selection was made for resistance to *Fusarium* or for any agronomic traits during the development of either population. Although initially 100 F_2 individuals were used to develop each population, the population numbers were reduced to 90 and 78 $F_{4:5}$ lines because of germination failure and seedling death of some F_2 and F_3 individuals.

Plate 1. K132 x MLB-49-89A plants at F_4 generation developed through single-seed descent from F_2 individual plants.

3.2.3 Layout of the Experiment

Recombinant inbred lines and their parents were evaluated for their reaction to *Fusarium* root rot at 28 days after planting (Mukankusi, 2007). The two populations ($F_{4:5}$ of K132 x MLB-49-89A and of K20 x MLB-49-89A) and their parents were evaluated for *Fusarium* root rot using a randomised complete block design with two replications in wooden trays measuring 0.74 x 0.42 x 0.115 m (Plate 2). Each experimental unit consisted of 14 plants per row, in 0.42 m long rows for each population, each tray having nine recombinant inbred lines, plus susceptible and resistant parents.

Plate 2. Wooden trays used as blocks within a replication, during the study.

3.2.4 Preparation of the Inoculum, Inoculation and Disease Evaluation

3.2.4.1 Source of Inoculum

Isolate FSP-3 of *Fusarium solani* f.sp. *phaseoli,* was used to prepare the inoculum. This had been previously identified as the most virulent *Fusarium* root-rot isolate in a study on common bean germplasm (Mukankusi, 2007). The isolate was obtained from infected bean fields in south-western Uganda and is maintained by CIAT (in Uganda). Pure colonies of the isolate, cultured on slants of Potato Dextrose Agar (PDA) (HiMedia Laboratories, Mumbai, India) at 5^{o}C, were sub-cultured to PDA plates for a period of up to 21 days and then used to prepare inoculum. As described below, infested sorghum seed was used as a medium for *Fusarium solani* f.sp. *phaseoli* inoculation in the soil (Mukankusi, 2007).

3.2.4.2 Inoculation Procedure

To sterilise sorghum seeds, 500 g of moistened kernels were autoclaved twice for one hour at 120^{o}C, using autoclave plastic bags. Thereafter, the sorghum seeds were allowed to cool before adding the isolate. One PDA plate of the FSP-3 isolate was mashed into 4-10 ml of sterile and deionised water to make slurry, which was then spread evenly onto the surface of the autoclaved sorghum kernels still in the autoclave bags. The plastics bags were resealed and agitated to mix the slurry with the sterilised sorghum and the culture left at room temperature for 14 days in order to colonise the sorghum kernels. Wooden trays in the screenhouse were partially filled to 2/3 capacity with sterilised loamy sand, containing 80% sand and 20 % loam soil (Mukankusi, 2007). The soil was amended with inorganic fertilizers NPK (1:1:1) at a rate of 3 x 10^{-3} kg·ml^{-1} , both before planting and 7 day later. The prepared inoculum was added to the soil at a rate of 500 g of infested sorghum kernels per tray. The infested kernel was thoroughly mixed with the soil in a tray to ensure an even distribution of inoculum. In order to increase disease pressure

immediately after inoculation, the susceptible variety K132 was grown in the trays for 28 days and then uprooted. Subsequently, the two mapping populations of test plants were planted. The plants were watered once a day until the day they were evaluated, to eliminate moisture stress and ensure adequate moisture for good disease development.

3.2.4.3 Procedure for Evaluation of Disease Reactions

Disease reaction was visually estimated at 28 days after planting. Six randomly selected plants from each recombinant inbred line in a row were uprooted, taking care not to disturb the main portion of the root system. Roots were cleaned of debris by washing the hypocotyls and roots in water. The levels of infection on the roots and hypocotyls were observed and disease severity estimated on each of the six plants per recombinant inbred line. The average disease severity score was obtained for each line, using the CIAT 1-9 scale (Abawi and Pastor-Corrales, 1990). In this system,

1= No visible symptoms;

3= Light discoloration, either without necrotic lesions or with less than 10% of the hypocotyls and root tissues covered with lesions;

5=Approximately 25% of the hypocotyls and root tissues covered with lesions, but tissues remaining firm, with some deterioration of the root system;

7=Approximately 50% of the hypocotyls and root tissues covered with lesions combined with considerable softening, rotting and reduction of the root system;

9= Approximately 75% or more of the hypocotyls and root tissues affected with advanced stages of rotting, combined with severe reduction in the root system.

3.2.6 Data Collection on Seed Attributes

Seed size and colour data were collected from the 73 F_4 lines of K20 x MLB-49-89A. The other 17 lines did not have enough seeds to record seed weights. Seed weight data was collected by taking 100 seed weight. The average moisture content of the seeds at the time of weighing was 10%, measured using GAC®500XT moisture meter (DICKY-john, Illinois, USA). Seed colour of the 90 lines was also visually assessed and recorded. There was no data collected on seed size and colour from K132 x MLB-49-89A population because of an inadequate amount of remnant seeds.

3.2.6 Statistical Analyses

The mean disease score for each recombinant inbred line of the two populations was calculated for each replicate and replicate means were used to compute the overall mean disease score for the line. The data were subjected to Analysis of Variance (ANOVA) using GenStat Discovery Edition 3 (Lawes Agricultural Trust, Rothamsted, U.K). Where significant differences were found, the means were compared using Fisher's Protected Least Significant Difference (LSD) test at P≤0.05 (Steel *et al.*, 1980). Correlation analysis was done using GenStat Discovery Edition 3 to establish any association between seed weight and *Fusarium* root rot disease severity index.

Broad sense (h^2_B) and narrow sense (h^2_N) heritabilities for *Fusarium* root rot resistance were estimated on a line-mean basis from two replications, using the expected mean squares generated from the analysis of variance table (Bernardo, 2002). Broad sense heritability was calculated based on the mean of the line as:

$$[\quad h^2_B = \frac{\sigma^2_G}{\sigma^2_G + (\sigma^2_e / r)} \quad] \text{..... (Equation 1)}$$

Where: σ^2_G = the genetic variance

σ^2_e = variance due to the environment

r = the number of replications.

Narrow sense heritability was calculated based on the mean of the line as:

$$[\ h^2_N = \frac{1.5\sigma^2_{A(F_2)}}{1.5\sigma^2_{A(F_2)} + (\sigma^2_e / r)}\ ] \text{..... (Equation 2)}$$

Where: $1.5\sigma^2_{A(F_2)}$ = additive genetic variance relative to the F_2 reference generation

σ^2_e = variance due to the environment

r = the number of replications

Assumption: In the equation for estimating narrow sense heritability, it was assumed that the non-additive genetic effects were negligible in the $F_{4:5}$ lines, because of their high level of homozygosity.

3.3 RESULTS

3.3.1 Disease Reactions to *Fusarium* Root Rot in $F_{4:5}$ K20 x MLB-49-89A Progeny

Significant genetic variation ($p \leq 0.01$) among the 90 $F_{4:5}$ recombinant inbred lines of K20 x MLB-49-89A population was observed for *Fusarium* root rot scores (Table 1). The mean root rot score for the population of recombinant inbred lines was 4.1, which was less than the mid-parent value of the two parents (5.4). Root rot scores for the recombinant inbred lines ranged from 1.8 to 8.8 (Table 2). Susceptible parent K20 had scores between 7 and 9 with a mean of 8.8, while scores for the resistant parent MLB-49-89A ranged from 1 to 3 with a mean of 2.3.

Table 1. Analysis of variance for *Fusarium* root rot scores of $F_{4:5}$ K20 x MLB-49-89A progeny.

Source of Variation	Degrees of Freedom	Mean of Squares	F-value
Replication	1	5.94	5.23*
Recombinant inbred line	89	8.61	7.64**
Residual	89	1.14	
Total	179		

*,**Significant at $p \leq 0.05$ and $p \leq 0.001$, respectively.

Table 2. Parents and progeny means, range and heritability estimates of *Fusarium* root rot resistance in 90 $F_{4:5}$ (K20 x MLB-49-89A) recombinant inbred lines.

Genotypes	*Fusarium* root rot score
Parents	
MLB-49-89A (Resistant Parent)	2.3
K20 (Susceptible Parent)	8.8
[a]Mid-Parent Value	5.6
Progeny	
Lowest	1.8
Highest	9
[b]Mean (90 $F_{4:5}$ lines)	4.1
[c]h^2_B (line-mean basis, 2 replications)	0.86
[d]h^2_N (referenced to additive variance in F_2)	0.81
[e]LSD ($P \leq 0.05$)	0.18
[f]CV (%)	26

Disease score was visually rated on a scale of 1-9. The scores are based on the CIAT 1-9 (Abawi and Pastor-Corales, 1990), where 1=very resistant and 9=very susceptible.
[a]Mid-Parent Value= Average value of the two parents.
[b]Mean of the 90 recombinant inbred lines.
[c]h^2_B = Broad sense heritability based on expected mean squares, line-mean basis from two replications, (*see text*, Equation 1) (Bernardo, 2002).
[d]h^2_N =Narrow sense heritability (based on an inbreeding coefficient of 0.5 between F_4 lines and assuming dominance and epistasis to be negligible) (*see text*, Equation 2) (Bernardo, 2002).
[e]LSD=Fishers Protected Least Significant Difference test, computed according to Steel *et al.* (1980).
[f]CV=Coefficient of Variation (Steel *et al.*, 1980).

The population was generally skewed towards resistance (Figure 1). Sixty seven (67) out of 90 lines scored less than 5, which is considered the threshold for resistance reaction. There was a continuous normal distribution observed among susceptible genotypes, while resistant lines were

strongly concentrated at a score of 2. Transgressive segregation towards both resistance and susceptibility was observed, but it was more pronounced toward resistance. Both broad and narrow sense heritability estimates were high. Broad sense heritability was estimated as 0.86, with narrow sense heritability h^2_N estimated as 0.81. Susceptible lines showed reduced root biomass compared to the resistant lines, which is one of the symptoms of *Fusarium* root rot infection (Plate 3).

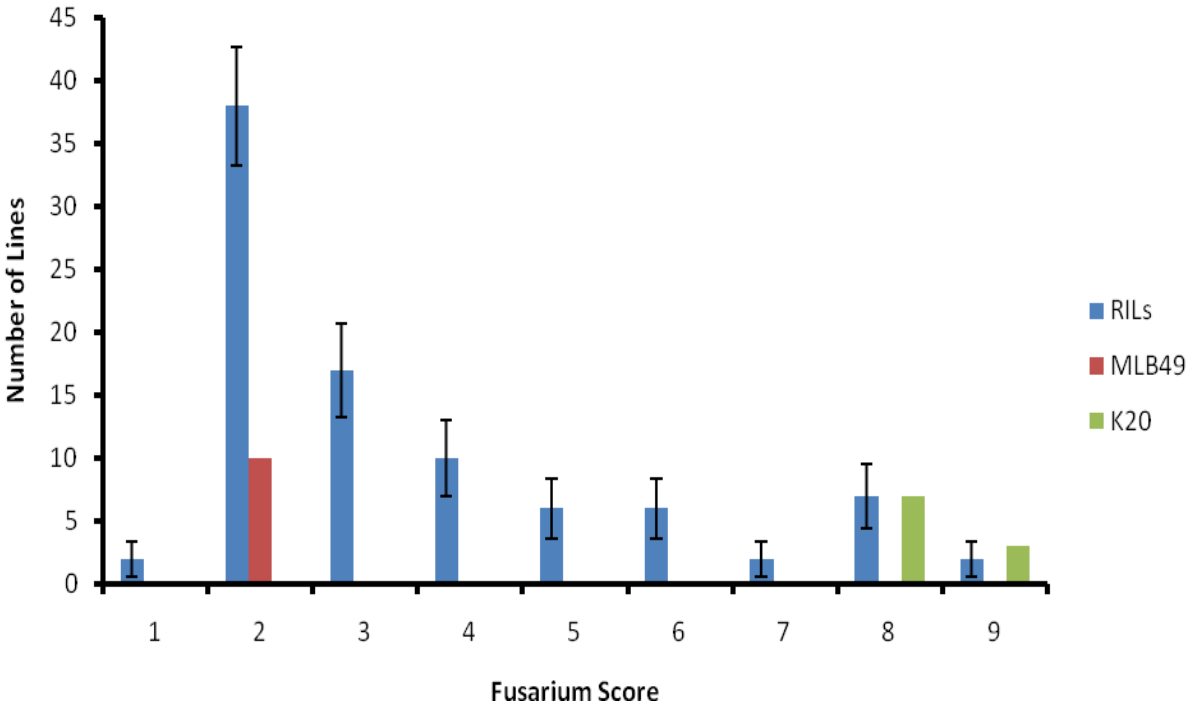

Figure 1. Frequency of *Fusarium* root rot ratings for a K20 x MLB-49-89A recombinant inbred line ($F_{4:5}$) population. MLB-49-89A is the resistant parent and K20 is the susceptible parent. Disease score was visually rated on a scale of 1-9 from CIAT, where 1=very resistant and 9=very susceptible (Abawi and Pastor-Corales, 1990).

Plate 3. Variation in levels of infection and extent of root growth on a susceptible recombinant inbred line compared to the two parents, K20 (susceptible) and MLB-49-89A (resistant).

3.3.2 Seed Weight in F_4 K20 x MLB 49-89A Progeny

The distribution curve for seed weight was consistent with a quantitatively inherited trait, skewed moderately toward small seed size, a characteristic of the Meso-american parent (MLB49-89A). with an average of 29.8 g (Figure 2).. The average seed weight was 29.8 g, with lines ranging from 21.4-45.2 g (Table 3). Parental seed was not available from plants grown in the same environment as the RILs, so no comparison could be made with parent seed weights. However, based on typical seed size for these two parents, it is likely that there was transgressive segregation for seed weight in both directions. Correlation analysis between *Fusarium* root rot and seed weight did not reveal any association between seed weight and *Fusarium* root rot scores (r = -0.009).

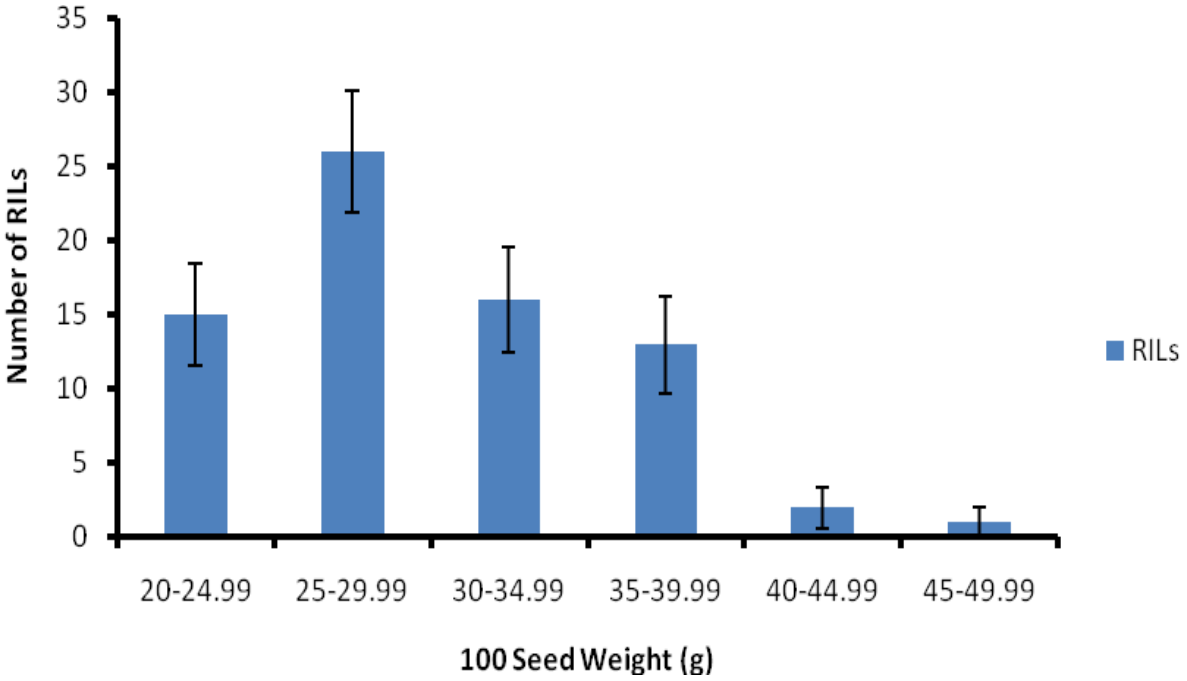

Figure 2. Frequency of seed weights for a population of F_4 lines of K20 x MLB-49-89A. MLB-49-89A is Meso-american and small-seeded, while K20 is Andean and large-seeded.

Table 3. Average seed weights for 73 F_4 lines of K20 x MLB-49-89A.

Genotype	Means of 100 Seed Weights (g)
Progeny	
[a]F_4 lines	29.8
Lowest	21.4
Highest	45.2
[b]CV (%)	19.6

[a]F_4 lines = Average seed weights of the 73 F_4 lines
[b]CV(%) = Percentage coefficient of Variation

3.3.3 Seed Colour in F_4 K20 x MLB 49-89A Progeny

There were generally fewer lines that had seeds with the black colour of MLB-49-89A (18 out of 73) than those that had the red mottled seed colour of K20 (23 out of 73) (Figure 3). There were 26 lines with a purple mottled seed colour. The purple colour is associated with grains that tend towards a red mottled colour. This segregation ratio suggests some form of epistasis for seed color.

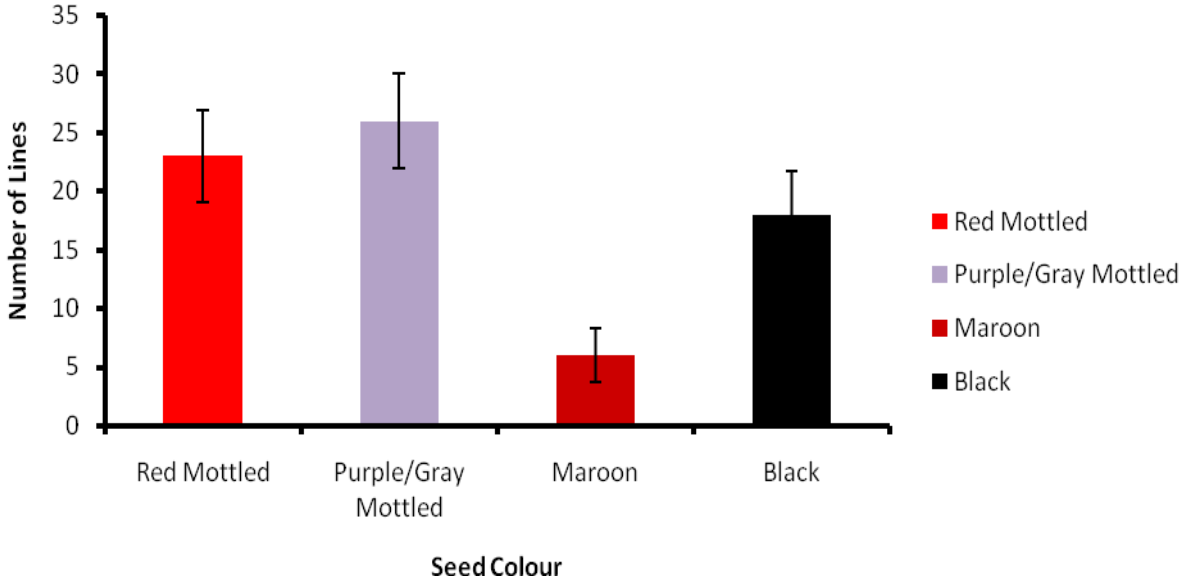

Figure 3. The frequency of seed colour for the F_4 lines of K20 x MLB-49-89A.

3.3.4 Disease Reactions to *Fusarium* Root Rot in $F_{4:5}$ K132 x MLB-49-89A Progeny

There was highly significant ($p \leq 0.001$) genetic variation for *Fusarium* root rot scores among the recombinant inbred lines in this population (Table 4). Scores for the recombinant inbred lines ranged from 1.1 to 9 with a mean of 5.3 (Table 5). The susceptible parent K132 had a mean score of 9 and the resistant parent, MLB-49-89A, had scores ranging from1 to 3 with the mean being 1.7 across trays. Both broad and narrow sense heritability estimates in the K132 x MLB-49-89A were very high. Broad sense heritability (h^2_B) was estimated as 0.98 while the narrow sense heritability (h^2_N) was estimated as 0.98.

For the K132 x MLB-49-89A population a total of 78 F_4 derived recombinant inbred lines were planted in 9 trays with two replications, but only 62 lines survived up to 4 weeks after planting. The other 16 lines died from *Fusarium* root rot; consequently no leaf tissue was collected for DNA extraction. These 16 lines were assigned a score of 9 to reflect their uniform susceptibility. The distribution exhibited a bimodal pattern with two peaks, one for resistance and one for

susceptible (Figure 4). There was, however, a normal distribution observed for genotypes within the resistant category (lines with a score less than 5).

Table 4. Analysis of Variance of the *Fusarium* root rot scores of K132 x MLB-49-89A $F_{4:5}$ progeny.

Source of Variation	Degrees of Freedom	Mean of Squares	F-Value
Replication	1	0.03	0.21
Recombinant inbred lines	61	17.26	102.36**
Residual	61	0.16	
Total	123		

**Significant ($p \leq 0.001$)

Table 5. Parents and progeny means, range and heritability estimates of *Fusarium* root rot resistance in 78 $F_{4:5}$ (K132 x MLB-49-89A) recombinant inbred lines.

Genotypes	Score
Parents	
MLB-49-89A (Resistant Parent)	1.7
K132 (Susceptible Parent)	9
[a]Mid-parent value	5.3
Progeny	
Lowest Progeny	1.1
Highest Progeny	9
[b]Mean (78 $F_{4:5}$ Progenies)	5.3
[c]h^2_B	0.99
[d]h^2_N	0.98
[e]LSD ($P \leq 0.05$)	0.2
[f]CV (%)	8

Disease score was visually rated on a scale of 1-9. The scores are based on the CIAT 1-9 (Abawi and Pastor-Corales, 1990), where 1=very resistant and 9=very susceptible.
[a]Mid-Parent Value= Average value of the two parents.
[b]Mean of the 90 recombinant inbred lines.
[c]h^2_B = Broad sense heritability based on expected mean squares, line-mean basis from two replications, (*see text*, Equation 1) (Bernardo, 2002).
[d]h^2_N =Narrow sense heritability (based on an inbreeding coefficient of 0.5 between F_4 lines and assuming dominance and epistasis to be negligible) (*see text*, Equation 2) (Bernardo, 2002).
[e]LSD=Fishers Protected Least Significant Difference test, computed according to Steel *et al.* (1980).
[f]CV=Coefficient of Variation (Steel *et al.*, 1980).

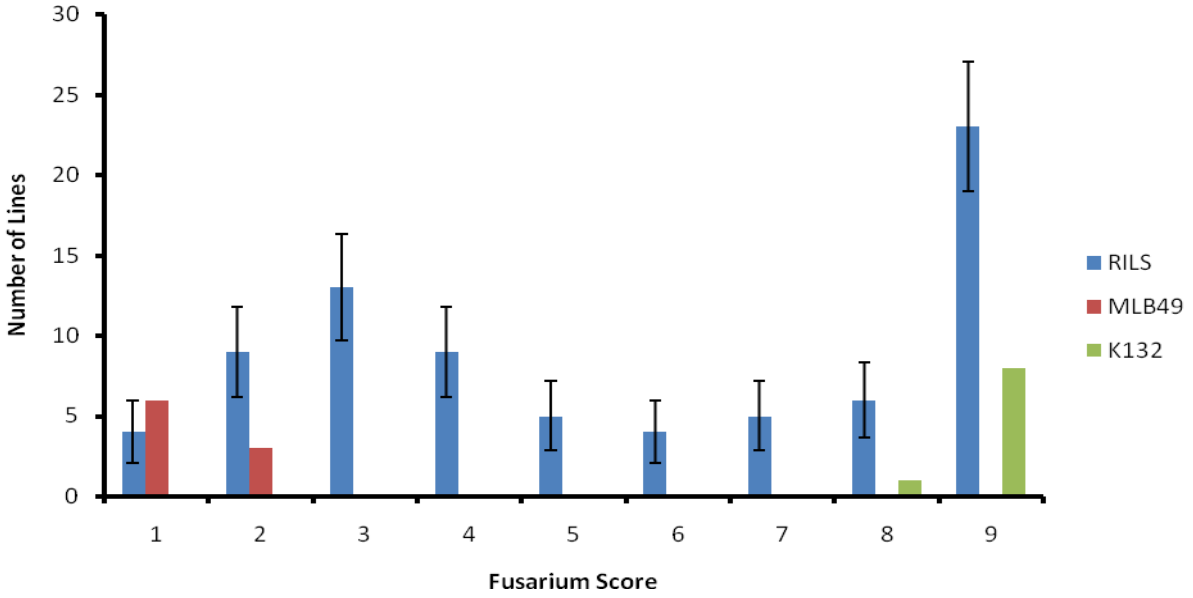

Figure 4. Frequency of *Fusarium* root rot ratings for a K132 x MLB-49-89A recombinant inbred line ($F_{4:5}$) population. MLB-49-89A is the resistant parent and K132 is the susceptible parent. Disease score was visually rated on a scale of 1-9 from CIAT, where 1=very resistant and 9=very susceptible (Abawi and Pastor-Corales, 1990).

3.4 DISCUSSION

The objective of this study was to confirm the usefulness of a Meso-american source (MLB 49-89A) to transfer resistance against *Fusarium solani* f.sp. *phaseoli* into locally adapted Andean types (K20 and K132). The results show that the Meso-american genotype MLB-49-89A is an effective source of resistance to *Fusarium* root rot, as indicated by the low to intermediate average scores for both populations (K20 X MLB-49-89A and K132 x MLB-49-89A). The average score of the K20 X MLB-49-89A population (4.1) was significantly lower than its mid-parent value (5.3), while that of K132 x MLB-49-89A was equal to its mid-parent value (5.3) and higher than the mean of the K20 x MLB-49-89A population. Some recombinant inbred lines of each population showed lower *Fusarium* severity scores than the resistant parent, indicating transgressive segregation for root rot resistance in both populations. Transgressive segregation has been reported in previous studies involving populations of inbred backcross lines developed from crosses between resistant Meso-american and susceptible Andean genotypes (Román-Avilés and Kelly, 2005). The transgressive segregants observed in this study for *Fusarium* root

rot offer hope for significantly improving this trait in the locally adapted Andean genotypes. Some of the resistant recombinant inbred lines in the current study are also large seeded. There was no correlation between seed weight and *Fusarium* root rot score in K20 x MLB-49-89A. Most of the recombinant inbred lines had a red mottled or purple seed colour. Selections from both K20 x MLB-49-89A and K132 x MLB-49-89A can produce lines that are not only resistant to *Fusarium* root rot but also possess good market attributes, such as large seeds and red mottled seed colour. Although the line used in the present study (MLB-49-89A) is not completely resistant to *Fusarium* root rot, it is an effective source that can be used to improve resistance in the Andean genotypes. Nevertheless environment tends to play a key role in disease development and severity of *Fusarium* root rot (Schneider *et al*., 2001). Overall, the scores obtained in this study for the resistant parent support the conclusion that resistant genotypes exhibit a consistent and substantial reduction in the disease incidence.

The average *Fusarium* severity scores on parents K20 and K132 were 8.8 and 9 respectively, indicating that both were highly susceptible. Inheritance of resistance to *Fusarium* root rot has been reported (Román-Avilés and Kelly (2005). In this study, the bimodal pattern observed in both populations suggests that at least one major gene may be contributing to resistance. Interestingly, progeny from K20 x MLB49-89A population was skewed towards resistance, with a majority of recombinant inbred lines having a disease severity score of less than 5, while the K132 x MLB49-89A was skewed to susceptibility, with many lines having a score of 9. Accordingly, these results suggest a complex pattern of inheritance with some quantitative and/or multi-gene inheritance in addition to at least one major gene. The results also clearly show differences between the parental effects of K20 and K132 on *Fusarium* root rot resistance. Such differences in means and distributions between the two populations suggest that K20 possesses one or more genes that interact in an epistatic manner with two or more resistance loci

in MLB-49-89A. In contrast, K132 apparently lacks the beneficial allele, or has an alternate allele that interacts towards susceptibility.

Previous studies (Mukankusi, 2007) have also shown MLB-49-89A to be an effective source of resistance to *Fusarium* root rot. In crosses involving resistant parents (MLB-49-89A, RWR719, Vunikingi, Umubano and MLB-48-89), with susceptible genotypes, crosses involving MLB-49-89A resulted in the lowest *Fusarium* severity scores. Consistent with the results of the present study, the cross of K20 x MLB49-89A had a lower average *Fusarium* severity score than did K132 x MLB49-89A in an earlier test involving the F_1 and F_2 generations (Mukankusi, 2007). The implication of these results is that although MLB49-89A is generally an effective source of resistance to *Fusarium* root rot, the degree of its effectiveness depends on the Andean parent genotype used. The results of this study have shown that it would be easier to improve K20 than K132 for *Fusarium* root rot resistance when using MLB-49-89A as a parent. In the USA, Silbergel (1987) developed a resistant large-seeded cultivar FR266 that belongs to the Andean gene pool, using a small and black-seeded variety, N203, as a source of resistance from the Meso-american gene pool. Cultivar FR266 has since been used successfully in crosses with beans from the Andean gene pool for improving resistance to *Fusarium* root rot (Schneider *et al.*, 2001). Other studies have shown that small and black-seeded Meso-american lines are in general more resistant to *Fusarium* root rot than the large, seeded varieties and are valuable sources of resistance (Beebe *et al.* 1981; Abawi and Pastor-Corrales, 1990).

In the current study, both the narrow and broad sense heritability estimates obtained for the K20 x MLB-48-89A (h^2_B=0.86; h^2_N=0.81) and K132 x MLB-49-89A (h^2_B=0.99, h^2_N=0.98) are very high (line mean basis, two replications). It is expected, that such heritability estimates derived from screenhouse experiments are substantially higher than field heritability estimates for a given population, since the objective for developing screenhouse evaluations is to reduce

environmental variation (Schneider *et al*., 2001). In addition to reducing environmental variation, screenhouse screening also reduces the interaction of genotypes with unintended effects of other biotic and abiotic factors that can occur under field conditions. In this study, screenhouse evaluations used a single isolate of *Fusarium solani* f.sp. *phaseoli*, precluding confounding effects from other soil-borne pathogens common under field conditions further reducing environmental variation. Hassan *et al*. (1971) reported broad sense heritability of resistance to *Fusarium* root rot varying from 61.5% to 64.3% under greenhouse conditions and 77.9% to 79.7% under field conditions while narrow sense heritability varied from 25.9% to 44.3% for inter-genepool crosses. Relatively high narrow sense heritability estimates for resistance to *Fusarium* root rot, ranging from 0.48 to 0.71 in the F_4-derived recombinant inbred lines developed within the same gene pool, have been noted in previous studies (Schneider *et al*., 2001). Narrow sense heritability estimates for *Fusarium* root rot resistance ranged from 0.10 to 0.51 for kidney beans and from 0.2 to 0.82 for the cranberry-inbred backcrossed populations (Román-Avilés and Kelly, 2005). In a previous study involving the same three parents as the present study, broad sense heritability on an F_2 single-plant basis ranged from 0.22 to 0.69 and narrow sense heritability was estimated at 0.34 (Mukankusi, 2007).

The occurrence of transgressive segregation for resistance in lines with desirable market traits, coupled with the high heritabilities obtained in this study and in previous studies, indicate that introgressing resistance into locally adapted Andean genotypes from Meso-american genotype MLB-49-89A should be possible. Since resistance to *Fusarium* root rot involves complex inheritance that interacts with the environment, approaches that reduce environmental variation, such as screenhouse screening, should be adopted to make selection more effective.

CHAPTER FOUR

MAPPING QUANTITATIVE LOCI FOR *FUSARIUM* ROOT ROT RESISTANCE IN COMMON BEAN.

4.1 Introduction

Genetic resistance to *Fusarium solani* f.sp. *phaseoli* is polygenically controlled and strongly influenced by environmental factors that confound the expression and detection of physiological resistance mechanisms. Since traits such as resistance to root rot are genetically complex and difficult to evaluate, the efficiency of phenotypic selection is low (Roman-Aviles and Kelly, 2005). A complex inheritance pattern, combined with cross-incompatibility, have limited attempts to transfer *Fusarium* root rot resistance into Andean bean genotypes, despite extensive information on sources of resistance in the Meso-american gene pool (Beebe *et al*., 1981). Since field selection is laborious and destructive sampling is needed to identify resistance, indirect selection for resistance to *Fusarium solani* f.sp. *phaseoli* based on DNA markers linked to the resistance Quantitative Trait Loci (QTL) should facilitate breeding progress. Using molecular markers linked to major QTL for *Fusarium solani* f.sp. *phaseoli* resistance may prove valuable. QTL-marker associations also may provide greater understanding of inheritance and mechanisms of quantitative disease resistance to more than one disease (Ariyarathne *et al*., 1999). The aim of this study was to identify significant phenotype-SSR (simple sequence repeat) marker associations that can be used to facilitate marker-assisted selection for *Fusarium* root rot resistance in common bean. The specific objective of this study was to identify quantitative trait loci conditioning resistance to *Fusarium* root rot in common beans.

4.2 MATERIALS AND METHODS

Mapping the QTL for *Fusarium* root rot resistance involved the following steps:

i. Developing a mapping population;

ii. Identifying candidate markers and screening them for polymorphism;

iii. Constructing a linkage map;

iv. Analyzing phenotype-marker associations, using single marker analysis and composite interval mapping.

4.2.1 Choice of Mapping Population

The main mapping population was derived from the K132 x MLB 49-89A. Additionally, analysis was based on the K132 x MLB 49-89A progeny. However, only 4 SSR markers were used for single marker analysis in K20 x MLB 49-89A progeny because of resource constraints. The choice of K132 x MLB 49-89A population is based on the fact that K132 is more susceptible to *Fusarium* root rot than K20. Also, K132 has a larger seed size than K20 and is currently more popular among farmers and consumers of beans in East Africa (Mukankusi, 2007). The two parents, K132 and MLB-49-89A, are also more highly genetically and phenotypically contrasting compared to K20 and MLB-49-89A.

4.2.2 Study Site and Population Development

The laboratory experiments in this study were conducted in the biotechnology laboratory in the Department of Crop Science of Makerere University, Kampala, Uganda. Although 100 F_2 of K132 x MLB-49-89A were planted to derive recombinant inbred lines, the final population was reduced to 78 $F_{4:5}$ lines because some F_2 and F_3 seeds failed to germinate and others died at the seedling stage. During evaluation, 16 of these lines died from *Fusarium* root rot before DNA could be extracted from them, leaving only 62 lines for QTL analysis. In K20 x MLB 49-89A, 90 $F_{4:5}$ lines were derived from an initial 100 F_2 plants. Both populations were developed as

described in section 3.2.2. The phenotypic data in section 3.3.4 was used to identify the QTL for *Fusarium* root rot in K132 x MLB-49-89A population while the data in section 3.3.1 was used to identify significant phenotype-marker associations in K132 x MLB-49-89A population.

4.2.3 Identification of Candidate SSR Markers

In-silico analysis was done to identify SSR markers that are likely to be linked to QTL that condition resistance to *Fusarium* root rot. Using RAPD markers, previous studies mapped QTL that condition resistance to *Fusarium* root rot to the linkage groups B2, B3 and B5 of the integrated bean map (Figure 1) (Freyre *et al*., 1998; Schnider *et al.*, 2001; Navarro *et al.*, 2004; Roman-Aviles and Kelly, 2005; Micklas *et al*., 2006). To date there are many SSR markers that have been identified and mapped in common bean and assigned to 11 linkage groups of the integrated genetic map. The starting point for this *in-silico* analysis was to identify SSR markers that have been mapped to linkage groups B2, B3 and B5 of the integrated linkage map of common bean.

4.2.4 Genomic DNA Extraction

Genomic DNA was isolated from all materials following the modified protocols of Dellaporta *et al*. (1983) and Vallejos *et al.* (1992). Fresh leaf tissue from each parent were harvested at the first trifoliate leaf stage. Frozen leaves from each parent were ground to a very fine powder in liquid nitrogen using a mortar and pestle. About 5 g of the powder was transferred to a 50 ml centrifuge tube and mixed thoroughly after adding 15 ml of hot (65^{o}C) CTAB extraction buffer [150 mM Tris-HCl pH 8, 15mM EDTA pH 8, 1.05 M NaCl, 1.5% CTAB, 1% PVP-40, 1.5% β-Mercaptoethanol]. The mixture was incubated in a water bath at 65^{o}C for 20 minutes with occasional vigorous shaking. Initial extraction used 15 ml of phenol: chloroform: octanol

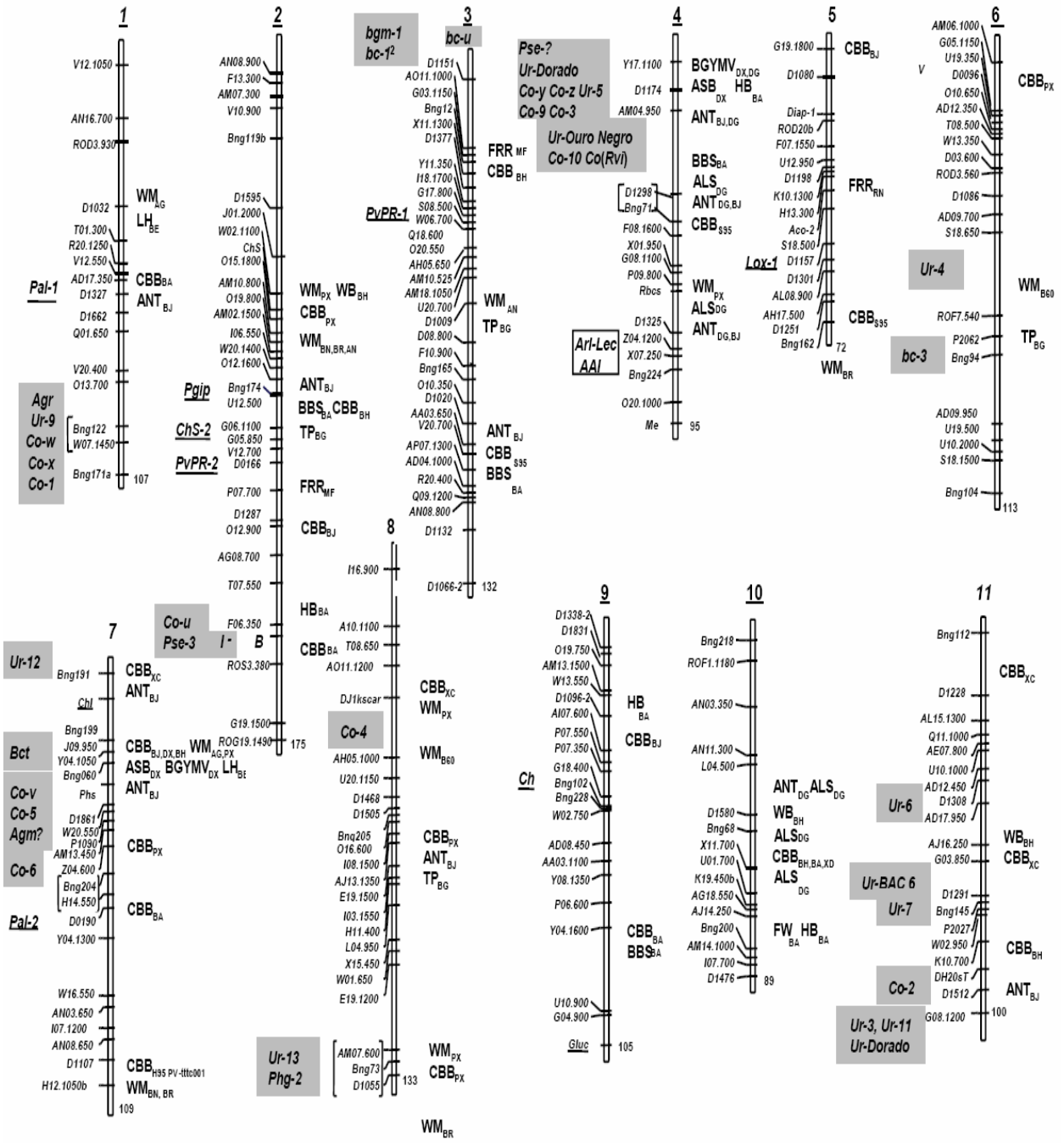

Figure 1: Comprehensive genomic map of disease resistance genes and QTL in common bean. *Fusarium* root rot QTL are mapped to B2, B3 and B5.

To the right of each linkage group are QTL mapped in various populations showing: ALS resistance to angular leaf spot, ANT anthracnose, ASB ashy stem blight, BGYMV bean golden yellow mosaic virus, BBS bacterial brown spot, CBB common bacterial blight, FRR *Fusarium* root rot, HB halo blight, LH leaf hopper, TP thrips, WB web blight and WM white mold resistance. Symbols in subscript represent the source population of the QTL. (Source: Miklas *et al.*, 2006)

(25:24:1) with the mixture shaken well until an emulsion was seen. The tubes were then centrifuged at 2880 g for 20 minutes at 4^{o}C to resolve the phases (Jouan BR4i Multifunction Centrifuge, Cadex, France). The upper aqueous phase was then transferred to a fresh tube to which an equal volume of chloroform: ethanol (24:1) was added and shaken well by inverting the tubes.

The tubes were then centrifuged again at 2880 g at 4^{o}C for 5 minutes to resolve phases. The aqueous phase (upper) was transferred to a fresh tube to which 0.1 volume of 3M sodium acetate (pH 5.2) and 0.6 volume of ice-cold isopropanol was added, mixed and then kept at -20^{o}C for 1 hour. The tubes were then centrifuged once more at 2880 g at 4^{o}C for 20 minutes to collect the precipitate. The supernatant was drained and the pellet washed with ice-cold 70% ethanol and left to dry at room temperature (for approximately 1 hour). Thereafter 500 µL of TE buffer (10:1) [10 mM Tris-HCl pH 8, 1mM EDTA pH 8] was used to dissolve. DNA concentration were measured using a NanoDrop ND-1000 Spectrophotometer (NanoDrop technologies, Delaware, USA). Each DNA solution was transferred to a 1.5 ml micro-centrifuge tube and stored at -20^{o}C until use. The DNA was diluted to 10 ng μL^{-1} for later use in Polymerase Chain Reactions (PCR).

4.2.5 PCR Amplification and Electrophoresis

A total of 35 SSR markers were selected (Appendix 1) and tested for polymorphism with K132 and MLB 49-89A. The primers were synthesised by the Molecular and Cell Biology laboratory of the University of Cape Town, South Africa. The PCR was performed in 20 µL final volume containing 50 ng of genomic DNA, 0.5 µM each of forward and reverse primers, 0.25 mM of dNTP mix, 1 unit of *Taq* polymerase (Promega, Madison, USA), 10 x *Taq* buffer and 2mM of $MgCl_2$. The reactions were carried out in a GeneAmp® PCR system 9700 thermocycler (Applied

Bio system), programmed for one pre-cycle at 94^{o}C for 1 minute, followed by 35 amplification cycles (94^{o}C for 1 minute, the specific annealing temperature of the primer pair for 1 minute and 72^{o}C for 1 minute), followed by a final extension at 72^{o}C for 7 minutes. The PCR products were loaded onto 5% w/v agarose metaphor gels [Seakam agarose (Cambrex, Rockland, USA)] in 1 x TBE buffer (0.83M Boric acid, 1M Tris-HCL and 10 mM EDTA) and run for 3 hours at 130 V using a BIO-RAD electrophoresis system (BIO-RAD Laboratories, California, USA). The DNA was visualised by ethidium-bromide staining (Promega, Madison, USA), using Gel Doc 1000 documentation system (BIO-RAD Laboratories, California, USA).

4.2.6 Genotypic Scoring of the Populations

The SSR markers that were found to be polymorphic between the susceptible parent (K132) and resistant parent (MLB49-89A) were used to genotype this mapping population of $F_{4:5}$ recombinant inbred lines. Total genomic DNA for each of the recombinant inbred lines was isolated from bulked leaf tissue of five screenhouse-grown plants per recombinant inbred line. An equal quantity of fresh leaf tissue from the five plants of each line was harvested at the first trifoliate leaf stage. Recombinant inbred lines carrying the allele from the susceptible parent at the polymorphic SSR loci were scored as 0, while those carrying the allele from the resistant parent were given a genotypic score of 2. Recombinant inbred lines carrying alleles from both parents (heterozygotes) were given a genotypic score of 1. The K20 x MLB-49-89A population was scored at the two SSR marker loci that had significant association with *Fusarium* root rot resistance in K132 x MLB-49-89A. Two of the SSR markers that did not show significant associations in K132 x MLB-49-89A population were also included.

4.2.7 Quantitative Trait Loci Analysis

To identify SSR markers that segregated normally in the mapping population, the Chi-square goodness-of-fit test of the observed segregation ratio for each marker was tested against the expected genotypic ratio of 1:1 for the population at $p \leq 0.05$ (Gomez and Gomez, 1984). Only the K132 x MLB-49-89A population was analysed to identify linkage groups of the SSR markers. The linkage groups were developed using MAPMAKER/EXP programme version 3.0 (Lander *et al.*, 1987). Linkage groups of the markers were determined by the group command of MAPMAKER/EXP at a LOD score of 2.0 and a maximum distance of 50 cM, using the Haldane units (Lander *et al.*, 1987).

4.2.7.1 Single-Marker Analysis

The relationship between molecular markers and phenotypic scores were first analysed by single marker analysis to identify SSR markers that had significant association ($p \leq 0.05$) with *Fusarium* root rot scores. In this population, QTL Cartographer version 2.0 was used for single marker analysis (Basten *et al.*, 2003), which uses the phenotypic and genotypic data in a simple linear regression model:

$$[\ Y_j = \beta_0 + \beta_{YX} X_j + \varepsilon_j\ ] \quad \text{(Equation 3)}$$

Where: Y_j = phenotypic value of the j^{th} recombinant inbred line

B_{YX} = regression coefficient for Y on $Xand$ is also the expected difference between the trait values in the two marker classes

X_j = indicator variable according to the marker genotype of the j^{th} line;

ε_j = random error term.

This model regresses the trait value on marker genotype. The null hypothesis of no linkage between the marker and the trait score was rejected if the regression indicated a significant slope

of trait value related to marker class. The *F* statistic compared the hypothesis H_0: $B_{YX} = 0$ to an alternative H_1: $B_{YX} \neq 0$ (Basten *et al.*, 2003). A *t*-test was used for single marker analysis in the K20 x MLB-49-89A population (Zar, 1998).

4.2.7.2 Composite Interval Mapping

After creating the linkage groups with MAPMAKER/EXP, QTL Cartographer version 2.0 for Windows was used to map composite intervals (Basten *et al.*, 2003), including only markers assigned to partial linkage groups. Permutation analysis was performed (1000 permutations) to identify the significance threshold of the test statistic (Logarithm of Odds) for individual QTL at $p \leq 0.05$ (Doerge *et al.*, 1997). The window size was set to 10 cM and the number of markers for background control was five. For composite interval mapping, the presence of a QTL was declared significant whenever the LOD score exceeded the threshold levels. The estimated position of the QTL was the point at which the maximum LOD score was found in the region under consideration.

4.3 RESULTS

4.3.1 Identification of Polymorphic SSR Markers between K132 and MLB49-89A

A total of 35 candidate SSR markers were identified from the bean linkage map (Appendices 2, 3 and 4) and tested for length polymorphism on K132 and MLB-49-89A, the parental lines susceptible and resistant to *Fusarium* root rot, respectively. Fifteen of the tested SSR markers, representing about 43% of the tested SSR markers exhibited fragment size polymorphism between the two parents (Plate 4 and 5). Only twelve of the fifteen polymorphic markers were used to genotype the mapping population because the polymorphic bands of the other three were not well distinguishable on agarose. Details of these markers are shown in Table 6. Five of the

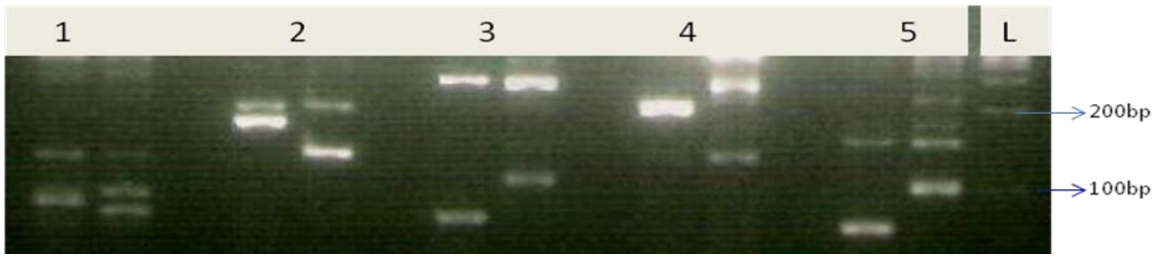

Plate 4. Gel picture of five polymorphic SSR markers. 1=Pv-gccacc001, 2=Pv-at006, 3=BM139, 4=BM156, 5=BM172 and L=100bp Ladder. The two lanes for each marker represent the parents, MLB-49-89A and K132.

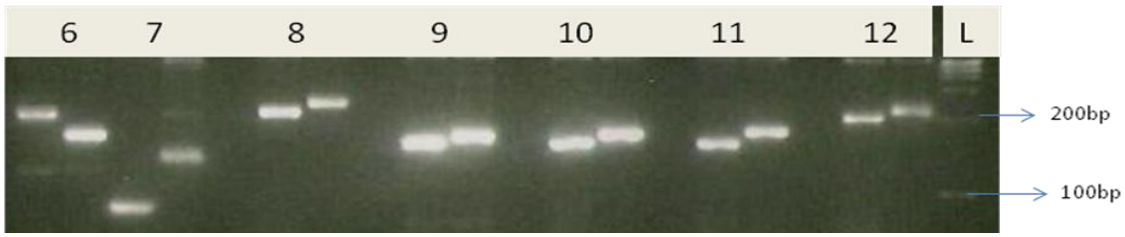

Plate 5. Gel picture of seven other polymorphic SSR markers. 6=BM167, 7=BM152, 8=PVBR61, 9=PVBR109, 10=PVBR8, 11=PVBRR255, 12=BM175 and L=100bp Ladder. The two lanes for each marker represent the parents, MLB-49-89A and K132.

polymorphic markers (Pv-gccacc001, BM139, BM152, BM156 and BM167) have been mapped to linkage group B2 of the consensus map. The other four (BM172, PVBR255, PVBR87, PVBR109) and three (Pv-at006, PVBR 61, BM 175) polymorphic markers have been mapped to linkage groups B3 and B5, respectively, of the bean consensus map (Appendices 1, 2 and 3).

4.2 SSR Marker Segregation in $F_{4:5}$ K132 x MLB-49-89A Progeny

When a Chi-square test was performed ($p \leq 0.05$), two of the twelve polymorphic marker loci deviated from the expected 1:1 segregation ratio (Table 7). Heterozygotes were not considered because their numbers were very few for each marker. The other 10 polymorphic loci had the expected segregation pattern. This represented segregation distortion for 16.6% of the SSR markers in this $F_{4:5}$ population. The two loci that had a skewed segregation ratio showed preferential transmission of paternal alleles (from MLB 49-89A). Plate 6 shows the segregation pattern of one of the polymorphic SSR loci in the $F_{4:5}$ mapping population.

Table 6. Twelve SSR markers polymorphic between parent genotypes K132 and MLB-49-89A.

Marker	Linkage group	Core Motifs	Fragment size (bp)*
PVBR87	B3	$(GA)_{16}$	163
PVBR109	B3	$(CT)_{16}(GT)_4$	150
PVBR255	B3	$(CT)_9(CA)_6$	177
PVBR61	B5	$(AG)_{32}$	212
BM172	B3	$(GA)_{23}$	107
BM175	B5	$(AT)_5(GA)_{19}$	170
BM167	B2	(GA)	165
BM156	B2	$(CT)_{32}$	267
BM152	B2	$(GA)_{31}$	127
Pv-at006	B5	$(AT)_5$	132
BM139	B2	$(CT)_{25}$	115
Pv-gccacc001	B2	$(GCCACC)_5$	95

*****Expected fragment size of the amplicon in base pairs. Includes only the 12 SSR markers that showed clear separation of the contrasting fragment size bands on agarose.

Table 7. Segregation pattern of the 12 polymorphic SSR marker loci in the $F_{4:5}$ K132 x MLB49-89A progeny.

Marker	Linkage Group	Progeny Segregation[a]	X^2(=3.84 at p=0.05)[b]
PVBR61	B5	24:33	1.42
PVBR87	B3	33:26	0.83
PVBR109	B3	36:22	3.38
PVBR255	B3	28:28	0.00
BM139	B2	22:33	2.20
BM152	B2	21:37	4.41*
BM156	B2	22:39	4.74*
BM167	B2	33:22	2.20
BM172	B3	26:28	0.07
BM175	B5	27:25	0.08
Pv-aat006	B5	34:26	1.00
Pv-gccacc001	B2	22:37	3.81

[a]The progeny are derived $F_{4:5}$ lines derived from K132 x MLB-49-89A. . The ordered pairs of numbers represent the frequency of lines homozygous for the K132 allele and the MLB-49-89A allele, respectively.

[b]Calculated Chi-square value (Gomez and Gomez, 1984) . The expected Mendelian segregation ratio is 1:1.

*Significant at 5% level of significance.

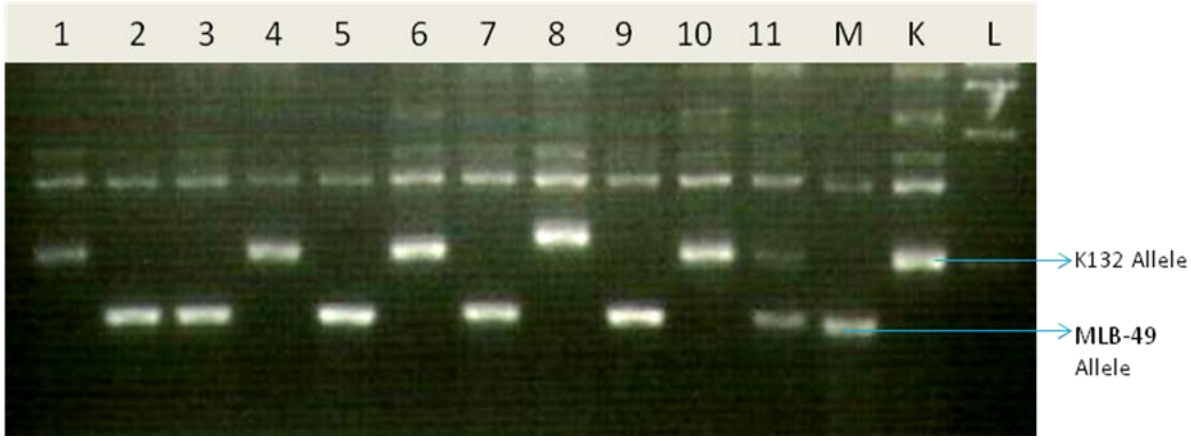

Plate 6. Segregation pattern of SSR loci BM172 in K132 x MLB-49-89A ($F_{4:5}$) population on agarose. Lanes 1 to 11 are recombinant inbred lines while M is MLB-49-89A, K is K132 and L is the 100bp Ladder.

4.4 Linkage Analysis of the SSR Markers

A linkage map was constructed using MAPMAKER/EXP version 3.0 by placing 9 of the 12 polymorphic SSR markers into three partial linkage groups (Linkage group 1, 2 and 3) (Figure 5). Each of the three partial linkage groups had three markers. The total length of these three partial linkage groups was 90.1 cM, with intervals between markers ranging from 0.9 cM to 28.9 cM. The other three SSR markers were not linked to any linkage group (Table 8). The three linkage groups represented only 7.5% coverage of the common bean genome, which has an estimated total size of 1200 cM.

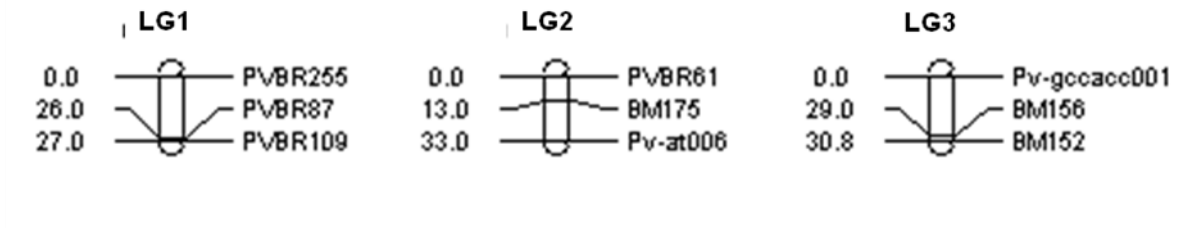

Figure 5. Three linkage groups of nine polymorphic SSR marker loci. Drawn using QTL Cartographer version 2.0 for Windows (Basten *et al.*, 2003). Linkage groups 1-3 correspond to the consensus map chromosomes B5, B3 and B2, respectively.

Table 8. Linkage group, marker interval and map distance of the 12 SSR markers

Linkage group	Marker Interval	Distance (cM)[a]
1(B5)	PVBR61 - BM175	13
1(B5)	BM175 - Pv-at006	20
2(B3)	PVBR255 – PVBR87	26
2(B3)	PVBR87 – PVBR109	0.9
3(B2)	Pv-gccacc001 – BM156	28.9
3(B2)	BM156 – BM152	1.8

[a]Map distances (Haldane units) computed using MAPMAKER/EXP version 3.0 (Lander *et al.*, 1987)
[b]Three SSR markers (BM139, BM167 and BM172) were not assigned to any linkage group

4.5 Mapping Quantitative Trait Loci for *Fusarium* Root Rot

4.5.1 Single Marker Analysis of Resistance in $F_{4:5}$ K132 x MLB-49-89A Progeny

The null hypothesis tested in the single-marker analysis is that the mean of the trait value is independent of the genotype at particular marker loci. A linear regression model was used to test this hypothesis and to detect any association between *Fusarium* root rot scores and the SSR markers. Among the twelve polymorphic SSR markers, single-marker analysis identified two markers closely linked to each other (PVBR87 and PVBR109) as significantly associated with *Fusarium* root rot scores ($p \leq 0.001$) (Table 9). Another marker, PVBR 255, had significant effects at $p \leq 0.05$ (Table 9). The other nine markers did not show any significant association with resistance to *Fusarium* root rot. Table 9 shows only nine markers analysed using linear regression of QTL Cartographer. The other three markers (BM139, BM167 and BM172) were analysed using *t*-test because they could not assigned to any linkage group; hence QTL Cartographer was not be used. The *t*-test result for these three markers showed no significant association ($p \leq 0.05$) of these markers with the *Fusarium* root rot resistance (Appendix 6). All three marker alleles associated with resistance to *Fusarium* root rot resistance in the current study came from the resistant parent MLB-49-89A. Single-marker analysis attributed a substantial proportion (34%) of the phenotypic variance to each of the two closely-linked markers that had the strongest association with *Fusarium* root rot scores. Due to the tight

linkage between these two markers (0.9 cM), multiple regression analysis also attributed the same R^2 value (0.34) to the two markers jointly as was indicated for each marker individually. This high R^2 value indicates that either of these two markers might be useful for marker-assisted selection.

4.5.2 Composite Interval Mapping of $F_{4:5}$ K132 x MLB-49-89A Progeny

Composite interval mapping eliminated marker PVBR255 as having an independent effect on *Fusarium* root rot, indicating that its association with root rot scores in single-marker analysis resulted from its linkage with two other markers on linkage group 1. A QTL was detected in the vicinity of markers PVBR109 and PVBR87, with a LOD score of 6.1 (Figure 6). The rapid decrease in LOD scores to the proximal side is an artefact of the large distance between PVBR87 and PVBR255. Without additional markers, the most that can be said about the location of the QTL is that it is much closer to PVBR109 and PVBR87 than it is to PVBR255. There were no QTL identified on the other linkage groups.

Table 9. Single Marker analysis: 62 $F_{4:5}$ lines scored for 9 SSR markers. Regression and *F* calculations are from QTL Cartographer version 2.0 (Basten *et al.,* 2003). Negative β values were contributed by the MLB 49-89A allele.

LG	SSR Marker	β_0[a]	β_{YX}[b]	*F*[c]	*pr (F)*[d]	R^2(%)[e]
1(B5)	PVBR255	5.32	-0.96	6.16	0.016*	12.9
1(B5)	PVBR87	4.93	-2.01	44.73	0.0001****	34.6
1(B5)	PVBR109	4.87	-2.01	43.23	0.0001****	34
2(B3)	PVBR61	5.26	0.36	0.83	0.367	
2(B3)	BM175	5.32	0.06	0.02	0.891	
2(B3)	Pv-at006	5.37	0.37	0.947	0.334	
3(B2)	Pv-gccacc001	5.38	-0.21	0.31	0.580	
3(B2)	BM156	5.45	-0.48	1.51	0.225	
3(B2)	BM152	5.39	-0.23	0.34	0.562	

The analysis fits the data to the simple linear regression model: $Y_j = \beta_0 + \beta_{YX}X_j + \varepsilon_j$

[a]Intercept of simple linear regression
[b]Slope of simple linear regression
[c]F statistic for testing that the marker is unlinked to the QTL by linear regression
[d]Probability value for the *F* statistic
[e]Coefficient of determination
*, **** Significant at the 5% and the 0.01% level of significance, respectively.

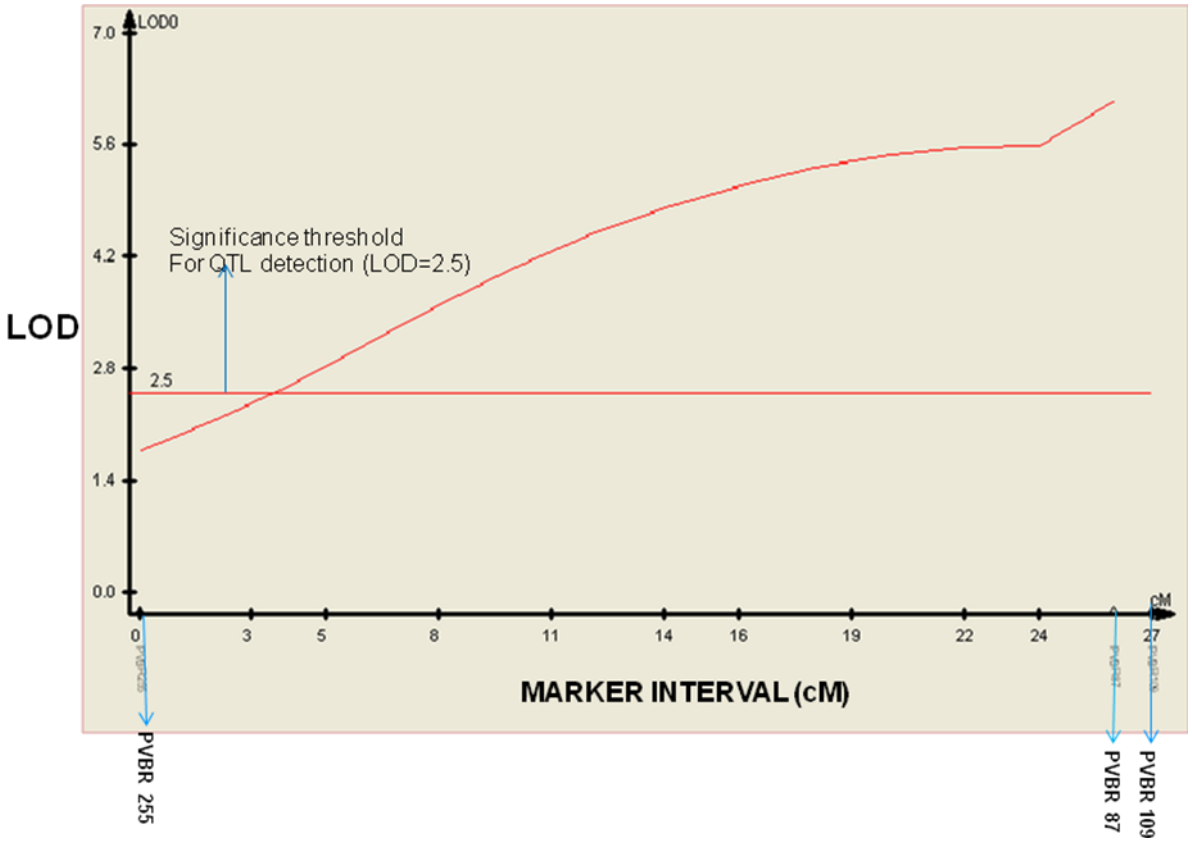

Figure 6. Composite Interval mapping analysis: A QTL was detected between PVBR87 and PVBR109 where there is the peak of the LOD score profile. The LOD score profile was drawn using QTL Cartographer version 2.0 (Basten *et al.*, 2003).

4.5.3 Single Marker Analysis of Resistance in $F_{4:5}$ K20 x MLB-49-89A Progeny

Four SSR markers were tested by single marker analysis, using a *t*-test to determine significance of the marker-phenotype association. The two SSR markers (PVBR87 and PVBR 109) that showed significant association with *Fusarium* root rot in K132 x MLB-49-89A population, also showed strong significant association ($p \leq 0.001$) in K20 x MLB-49-89A population (Table 10). The other two markers, BM156 and BM172, did not show significant association at $p \leq 0.05$. The coefficient of determination (R^2) values for the two closely linked SSR markers (PVBR109 and PVBR87) with significant effects was moderate, with single-marker analysis of each accounting for 14% of the phenotypic variance for *Fusarium* root rot score. Joint regression jointly attributed the same value to this pair of markers ($R^2 = 0.14$), because the two markers are tightly linked.

Table 10. Single Marker analysis: 90 $F_{4:5}$ lines of K20 x MLB-49-89A scored for 4 SSR markers.

SSR Marker	Progeny Segregation[a]	X^2(=3.84 at p=0.05)[b]	Difference between means[c]	*t*-value[d]	R^2(%)[e]
PVBR87	40:45	0.294	-1.62	-3.663***	14
PVBR109	40:45	0.294	-1.62	-3.663***	14
BM156	38:36	0.014	-0.07	-1.939	
BM172	27:44	4.070*	-0.94	-0.131	

[a]The progeny are derived $F_{4:5}$ lines derived from K132 x MLB-49-89A. The ordered pairs of numbers represent the frequency of lines homozygous for the K132 allele and the MLB-49-89A allele, respectively.
[b]Chi-square value (Gomez and Gomez, 1984). The expected Mendelian segregation ratio is 1:1.
[c]Difference between the phenotypic means of the two groups of lines: the group carrying the susceptible parent allele minus the group carrying the resistant parent allele.
[d]*t*-value statistic (Zar, 1998). The statistic tests association between markers and *Fusarium* root rot scores
[e]Coefficient of determination.
*, ***Significant at the 5% and the 0.1% level of significance, respectively.

4.4 DISCUSSION

The objective of this study was to map the quantitative trait loci for *Fusarium* root rot in a population derived between an Andean genotype, K132 and Meso-american genotype, MLB-49-89A. This study involved two main activities: (i) Screening SSR markers for polymorphism (ii) Mapping quantitative trait loci for *Fusarium* root rot resistance. Results of these activities are discussed below.

4.6.1 Level of SSR Marker Polymorphism between K132 and MLB-49-89A

Overall the SSR polymorphism between K132 and MLB49-89A identified in this study was relatively high (12/38=42.9%), but lower than that observed for some inter-gene pool parental combinations in which the polymorphism was as high as 60% (Bair *et al.,* 2006). A report on 264 microsatellites showed 43% polymorphism between Andean and Meso-american genotypes (Grisi *et al.,* 2007), a level similar to the current study. This relatively high level of polymorphism was expected in this study, considering that the two parents (K132 and MLB-49-89A) are from two different gene pools and highly contrasting for a number or traits (Grisi *et al.,*

2007). Conversely, the polymorphism levels tend to be lower in crosses among genotypes from within the same gene pool (Frei *et al*., 2005; Blair *et al.* 2006).

Although linkage group B2 had a relatively high number of polymorphic SSR markers, their distribution on the genetic map is not very good because markers BM139 and BM167 map very close to each other. BM156 and BM152 are the other two SSR markers that map very closely to each other on linkage group B2 of the bean genetic map. The implication of this uneven distribution of markers is that large gaps without a marker remained on each of the three linkage groups of the bean consensus map were targeted by this study. This scenario would diminish the chances of identifying QTL from these linkage groups. Adequate marker coverage of the common bean genome is critical to the detection and accurate mapping of QTL (Collard *et al*., 2005).

4.6.2 SSR Marker Segregation in the $F_{4:5}$ K132 x MLB-49-89A Progeny

Two of the 12 polymorphic marker loci deviated from the expected 1:1 segregation ratio in the $F_{4:5}$ mapping population. It is not unusual to find distorted segregation ratios in populations where a large number of markers have been analysed. For example, 105 of 599 marker loci (18%) used for the core bean genetic map showed a deviation from the expected segregation ration of 1:1 (Fryre *et al.*, 1998). Also, distorted segregation in 22% of SSR markers was reported in another study of common bean (Grisi *et al.*, 2007). In contrast, Yu *et al* (2000) found that all 16 SSR markers used in a study of F_7 recombinant inbred lines segregated according to the expected 1:1 ratio. Whenever many markers are screened, there is a higher likelihood of finding preferential transmission of either paternal or maternal alleles to the progeny, distorting the segregation ratio (Grisi *et al*., 2007).

4.6.3 Mapping Quantitative Trait Loci for Resistance to *Fusarium* Root Rot

Results from both single marker analysis and composite interval mapping indicate linkages of markers PVBR87 and PVBR109 (closely linked to each other) to a major QTL for *Fusarium* root rot resistance in the population from K132 x MLB 49-89A. The QTL identified on linkage group B3 of the consensus map could be considered major due to the high R^2 (34%) value and a LOD score of 6.1. The bimodal frequency distribution of the phenotypic scores in this study also points to a major gene for resistance in the K132 x MLB-49-89A and the susceptible and resistant frequency peaks are strongly associated with the K132 and MLB-49-89A alleles at these marker loci. Further studies are needed to accurately establish the chromosomal location of this major genetic factor. The small population size (n = 62) may have generated an upward bias in QTL effect estimation (Melchinger *et al*., 1998). Also, the large effect QTL might actually be a series of linked QTL, each of small effect (Flint and Mott, 2001), hence the need for fine mapping of such genomic regions.

The two SSR markers (PVBR87 and PVBR109) that are significantly associated with *Fusarium* root rot resistance in K132 x MLB-49-89A population also showed significant associations (R^2 = 14%, $P < 0.001$) in the K20 x MLB-49-89A population. This is a confirmation of the presence of a QTL identified on B3 close to these two markers in the K132 x MLB-49-89A population. Whereas a significant association was detected for the two markers, their overall contribution to the phenotype in the K20 x MLB-49-89A progeny was relatively low (R^2=14%) compared to the one in K132 x MLB-49-89A (R^2=34%) progeny. This result suggests the role of parent background effects. Results in chapter 3 of this thesis showed K20 x MLB-49-89A progeny population to have a lower disease severity index than K132 x MLB-49-89A progeny population. This result was attributed to the parental effect of K20 on resistance to *Fusarium* root rot resistance (*See* chapter 3). The significant associations of these two markers in two different

genetic background also shows that the QTL detected in K132 x MLB-49-89A population is stable in different genetic backgrounds. Stability of QTL in different genetic backgrounds is important in marker-assisted selection because it improves the usability of the markers in different genetic background (Collard, 2005).

It is interesting to note that the one major QTL identified in this study maps close to the region on linkage group B3 where QTL and major genes for resistance to anthracnose, common bacterial blight and bacterial brown spot have been mapped on the core map i.e., on linkage group B3 between RFLP markers D1020 and D1132 (Micklas *et al*., 2006). D1020 and D1132 are RFLP markers and were not included in the current study. Their position relative to the SSR markers used in this study is based on the available genetic maps of common bean. However, it should be noted that the QTL identified in this study is in a different location from the QTL for *Fusarium* root rot identified in previous studies (Micklas *et al*., 2006). That QTL was close to the *PVPR-1* gene location on the core map (Micklas *et al*., 2006).

The results of this study corroborate the importance of a segment of B3, where a QTL for resistance to *Pythium ultimum* and *Aphanomyces euteiches* was identified previously by Navarro et al. (2008), close to the QTL for *Fusarium* root rot identified in this study. In the study by Navarro *et al.,* (2008), the markers 13.800 and AP7.1000 that flanked the QTL for resistance to *Pythium* and *Aphanomyces* root rots were found between D1020 and D1132 of the integrated linkage map by Yu *et al*., (2000). This is the same region in which the SSR markers identified in the current study are located, as are the markers mentioned above for resistance to anthracnose, to common bacterial blight and to bacterial brown spot. The proximity of QTL for resistance to these several diseases suggests similar defence response genes or resistance mechanisms. Previous findings also suggest clustering of resistance genes within the common bean genome (Kelly *et al.,* 2003).

Detecting only one QTL for *Fusarium* root rot in the current study is not surprising. In a previous study, using RAPD markers and interval mapping, two QTL for *Fusarium* root rot were detected with LOD scores of 8 and 5 and with R^2 values of 30% and 20%, respectively (Chowdhury *et al.,* 2002). Unfortunately, the position of the linked markers on the consensus map was not reported. A single major QTL for *Fusarium* wilt resistance was detected directly at marker U20.750 with a LOD score of 23.9 and accounted for 63.5% of the phenotypic variance for disease score (Fall *et al.*, 2001). Other studies have detected a relatively large number of QTL for *Fusarium* root rot. Schneider *et al.* (2001) identified 16 QTL for resistance to *Fusarium*, using $F_{4:5}$ recombinant inbred lines, but none of the individual RAPD markers explained more than 15% of the phenotypic variation. In a different study, nine QTL that were significantly associated with *Fusarium* root rot resistance in the field and greenhouse each explained 5 to 53% of the total phenotypic variability (Román-Avilés and Kelly, 2005). In that study, the QTL associated with root rot resistance were located on linkage groups B2 and B5 of the integrated bean map (Román-Avilés and Kelly, 2005). In the current study, no QTL was detected on linkage group B2 or B5, but that is not surprising, considering the small population size and investigating only three markers covering only a portion of the each of these two linkage groups.

Overall, this study, using SSR markers, has identified a major QTL on linkage group B3. Two closely linked SSR markers were strongly associated with this major QTL. These two SSR markers showed significant association to *Fusarium* root rot resistance in two different genetic backgrounds. This result that confirms that the QTL identified in the K132 x MLB-49-89A population is indeed stable and the associated SSR markers may be useful for marker –assisted selection. Furthermore, the result provide good prospects for using approaches based on quantitative trait loci to introgress resistance to *Fusarium* root rot from Meso-american genotypes to locally adapted Andean bean genotypes.

CHAPTER FIVE

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 General Discussion

The general objective of this thesis was to develop approaches based on quantitative trait loci for introgressing resistance to *Fusarium* root rot from Mesoamerican sources into locally adapted Andean bean genotypes. Two $F_{4:5}$ populations (K20 x MLB-49-89A and K132 x MLB-49-89A) were used to investigate the effectiveness of a Mesoamerican line (MLB-49-89A) as a source of resistance to *Fusarium solani* f.sp. *phaseoli* when crossed with Andean varieties (K20, K132). K20 x MLB-49-89A had a lower average score compared to the mid-parent, while the average score for K132 x MLB-49-89A was equal to the mid-parent. Some recombinant inbred lines from each population showed a lower *Fusarium* severity score than the resistant parent, evidence of transgressive segregation for root rot resistance in both populations. The transgressive segregants observed in this study for *Fusarium* root rot offer hope for significantly improving this trait in common bean. Some of the recombinant inbred lines that were resistant are also large-seeded and most of the recombinant inbred lines had a red mottled or purple seed colour. These two characteristics are preferred in the Ugandan market. There was no correlation between seed weight and *Fusarium* root rot score. Selections from both K20 x MLB-49-89A and K132 x MLB-49-89A could produce lines that are not only resistant to *Fusarium* root rot but also possess good market attributes, such as large seeds and red mottled seed colour. Some of the resistant recombinant inbred lines developed in this study can be used as parents in breeding programmes to improve resistance to *Fusarium* root rot in the large-seeded Andean bean varieties that are popular in Uganda.

The population mean score for *Fusarium* root rot was much lower (4.1) for K20 x MLB-49-89A than was the population mean for K132 x MLB-49-89A (5.3). Generally, K20 x MLB49-89A was skewed towards resistance, while the K132 x MLB49-89A was skewed towards susceptibility. The distribution of *Fusarium* scores in the $F_{4:5}$ derived lines appears consistent with K20 possessing one or more genes that interact epistatically and beneficially with two or more loci for resistance from MLB-49-89A. In contrast, it appears that K132 either lacks this gene or possesses alleles that interact epistatically toward susceptibility. The implication of this result is that although MLB49-89A is generally an effective source of resistance to *Fusarium* root rot despite being only moderately resistant, the degree of its effectiveness depends on the Andean parent genotype used. Thus, it appears it would be easier to improve K20 than K132 for *Fusarium* root rot resistance when using MLB-49-89A.

In the current study, both the narrow and broad sense heritabilities obtained for the K20 x MLB-48-89A (h^2_B=0.86; h^2_N=0.81) and K132 x MLB-49-89A (h^2_B=0.99; h^2_N=0.98) are very high (reported here on a line-mean basis from 2 replications). The heritability estimates obtained in both this study and previous studies indicate that introgressing resistance to Fusarium root rot into Andean varieties from Mesoamerican line MLB-49-89A should be possible. However, screenhouse screening should be used for at least part of the testing in order to reduce environmental variation and make selection more effective.

Fifteen of the 35 SSR markers screened were polymorphic for fragment size (43%). Twelve markers with clearly differentiated bands were tested for association with resistance to *Fusarium* root rot. Significant *Fusarium* root rot QTL-SSR marker associations have been identified in this present study. Two closely linked SSR markers (PVBR87 and PVBR109) each showed a highly significant association with *Fusarium* root rot scores ($p \leq 0.0001$) in single marker analysis. Composite interval mapping in K132 x MLB-49-89A population detected a major QTL

on linkage group B3 of the consensus map, near PVBR87 and PVBR109. This QTL had an LOD score of 6.1 and coefficient of determination (R^2) of 34%, indicating it as a major QTL for *Fusarium* root rot resistance, even considering that R^2 may be overestimated due to the small population size (n=62). Single marker analysis in K20 x MLB-49-89A population also identified SSR markers PVBR87 and PVBR109 as having significant associations with *Fusarium* root rot resistance, with an R^2 of 0.14. While further studies are needed to establish the chromosomal location of this major genetic factor more precisely, it is probable that the QTL identified here is responsible for the bimodal distribution obtained for *Fusarium* root rot scores. The significant associations of the two markers with *Fusarium* root rot resistance in two different genetic backgrounds is a confirmation of the importance of the identified QTL. The result also shows that the identified QTL is stable in at least two different genetic backgrounds. QTL's that are stable in different genetic background with large effects such as the one identified in this study are useful starting points for marker-assisted selection for *Fusarium* root rot. SSR markers PVBR109 and PVBR87 are good candidates for further investigation on the prospects of using marker-assisted selection to introgress resistance to *Fusarium* root rot into the locally adapted Andean genotypes.

5.2 Conclusion

This study has demonstrated the potential of using Mesoamerican genotypes as sources of resistance to *Fusarium* root rot to improve locally adapted susceptible Andean genotypes and has also improved knowledge of the genetic basis of resistance to *Fusarium* root rot. A major quantitative trait loci for resistance to *Fusarium* root rot has been detected. The two SSR markers linked to this QTL are stable in two different genetic backgrounds. The results of this study has demonstrated good prospects for using QTL-based approaches to introgress resistance to *Fusarium* root rot from Mesoamerican genotypes into locally adapted Andean bean genotypes.

5.3 Recommendations

There is need to investigate the possible linkage or pleiotropy of this QTL on other agronomic traits. The parents used here would be appropriate for such a study, since K132 and K20 contrast strongly with MLB-49-89A for seed size, growth habit, days-to-flowering and maturity. There is also a need to determine whether the major QTL that has been identified in this study is also present in different genetic backgrounds and whether the two associated SSR markers are useable for marker-assisted selection in a wider range of materials. Fine-resolution mapping could be achieved using additional markers near the identified markers. More accurate determination of the QTL position could be useful for marker-assisted selection and might reveal whether the major effect QTL detected in this study is indeed a single QTL or is made up of several linked QTL, each with a small effect. The small numbers of SSR markers in this study were purposely targeted to chromosomal regions of anticipated effect. The population size was also small, so it is likely that other undetected QTL also exist. In view of the potential benefits, it is recommended that further studies be conducted involving a larger population size, additional markers near the identified QTL and a much larger number of SSR markers well distributed throughout the genome.

5.4 Future Perspectives

The ultimate goal of any plant breeding programme is to efficiently introgress targeted genomic regions with minimal linkage drag. To achieve this there is need to characterise genic regions beyond statistics such as QTL. In the case of this study and as a transition from QTL analysis to gene discovery, there is need for focusing on identifying the candidate gene(s) underlying the mapped QTL. Advances in genomics, bioinformatics and proteomics offer opportunity to achieve this. For example, by undertaking gene expression profiling gene(s) associated with resistance to *Fusarium* root rot could be identified and their relationship to the mapped revealed.

Microarray analysis or other global transcript profiling tools could also be used. These could be based on available data from whole legume genomes and other dicotyledonous species that have been studied. The availability of whole genome sequences of related species such as *Medicago sativa*, which is a model plant for legumes, offers an opportunity to identify genetic factors within a QTL. Candidate genes underlying QTL effects could be obtained by studying colinearity relationships between model plant like *Medicago sativa* and common beans, making the former the reference species for the identification of candidate genes. Through the identification of the *Medicago sativa* genomic regions that are collinear to the bean QTL region, inferences could be drawn on the genetic factors within the QTL. Eventually the common bean genome will also be sequenced a move that will provide ultimate resource of candidate genes for QTL mapping and cloning.

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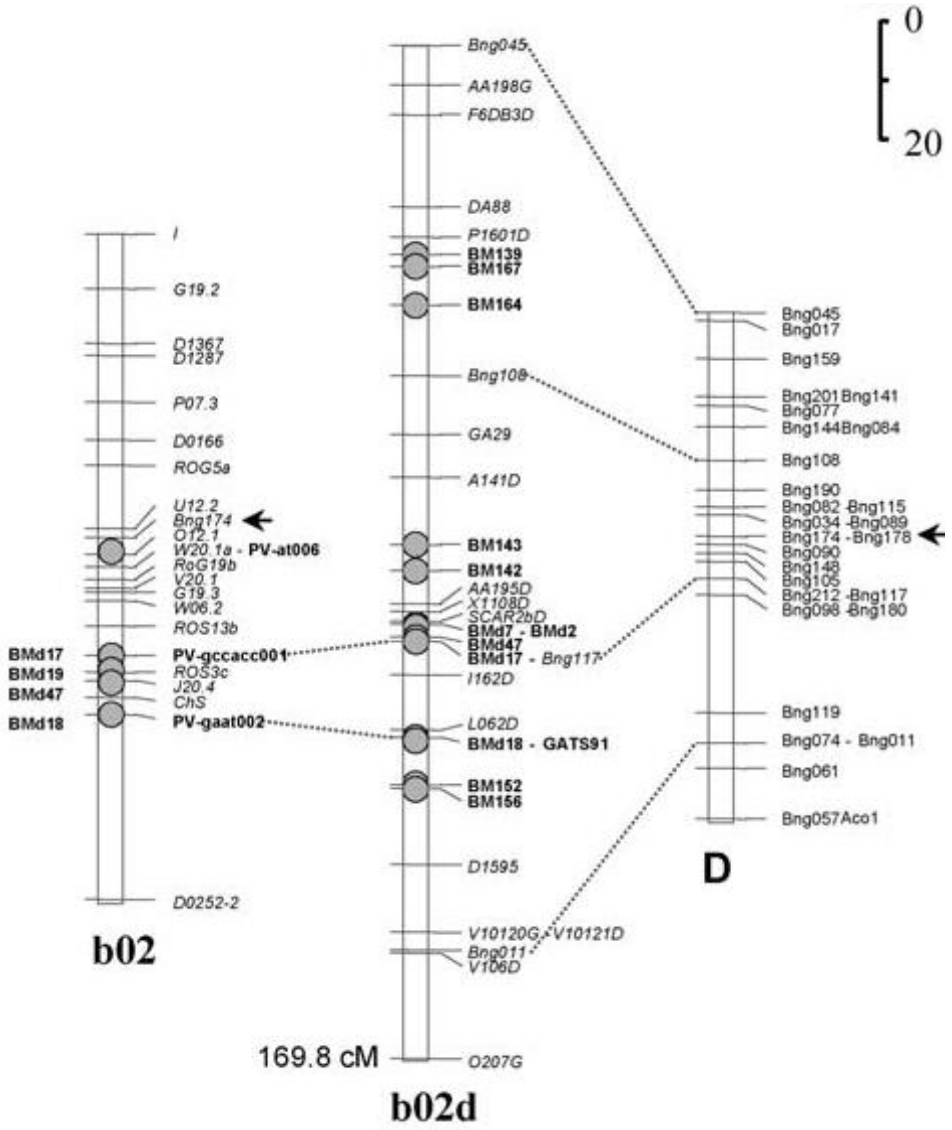
APPENDICES

Appendix 1: List of 35 SSR markers selected from the three linkage groups B2, B3 and B5 and screened for polymorphism.

Linkage group	SSR Marker	Size	Primer sequence
B5	PVBR236	232	TGT GAC GGA TCT CGT TGA AG TGT CCC GAT TCA TTG ATG TG
B5	PVBR93	171	TGG GGT GAG AGA GAA AGG TG TAC CAT AGC AGG CGT TGT TG
B5	PVBR69	210	GAT GCT GGC CTC TTT GTA GC CAC CCC TCT GTG TTT TTC TCC
B5	PVBR61	212	GAT GCT GGC CTC TTT GTA GC CAC ACC CCT CTG CTT TCT TC
B3	BM98	247	GCA TCA CAA AGG ACT GAG AGC CCC AAG CAA AGA GTC GAT TT
B2	BM167	165	TCC TCA ATA CTA CAT CGT GTG ACC CCT GGT GTA ACC CTC GTA ACA G
B2	BM164	182	CCA CCA CAA GGA GAA GCA AC ACC ATT CAG GCC GAT ACT CC
B2	BM143	143	GGG AAA TGA ACA GAG GAA A ATG TTG GGA ACT TTT AGT GTG
B2	GATS91	229	GAG TGC GGA AGC GAG TAG AG TCC GTG TTC CTC TGT CTG TG
B2	BM152	127	AAG AGG AGG TCG AAA CCT TAA ATC G CCG GGA CTT GCC AGA AGA AC
B2	PV-gccacc001	95	CGT TAG ATC CCG CCC AAT AGT CCG TCC AGG AAG AGC GAG C
B2	PV-gaat002	167	AAA CAC ACA AAA AGT TGG ACG CAC TTC GTG AGG TAG GAG TTT GGT GG
B2	PV-at006	132	CCG TTG CCT GTA TTT CCC CAT CGT GTG AAG TCA TCT GGA GTG GTC
B2	PV-cct001	149	CCA ACC ACA TTC TTC CCT ACG TC GCG AGG CAG TTA TCT TTA GGA GTG
B3	PV-at008	161	AGT CGC CAT AGT TGA AAT TTA GGT G CTT ATT AAA ACG TGA GCA TAT GTA TCA TTC
B3	VA-ag001	163	GGG TAG TAA AGG AAA GAG AAG AAA GAG CCA CCT TCT CGT ACT GTT CCA TG
B3	PVBR87	163	CTC ATT GCG TCT ACC AGT GC CCT AGG TTC CGC AGC ATG T
B3	PVBR109	150	GGC TGG AAA ACT ACC AAT GC CGC TAT TGT CGT GCA GTT TC
B3	PVBR131	198	GCG TCT GAG GAG AAG GAG GT CTC CCA ATC TCA CCA AAA CC
B2	Bmd-7	166	GGA TAT GGT GGT GAT CAA GGA CAT ACC CAA TGC CAT GTT CTC
B2	Bmd-18	156	AAA GTT GGA CGC ACT GTG ATT TCG TGA GGT AGG AGT TTG GTG
B5	PVBR82	182	CCC AAA GAG AAT GCA AGG TT GCT TCC CTT TCA ACG ACA TC
B2	PVBR106	209	CAA CAA ACA AGG CTG AAA AAC A AAA AAG AGA GGA GAG AGA AGA GAG C
B5	PVBR124	162	CCT AAA AAC CAG GTG CGA GA TGG GAA ACC TAG CCA AAC AC
B3	PVBR235	188	CAC GGT GAA CCA GAG TCT CA CCA CGA CTC TCT TGC TCT TG

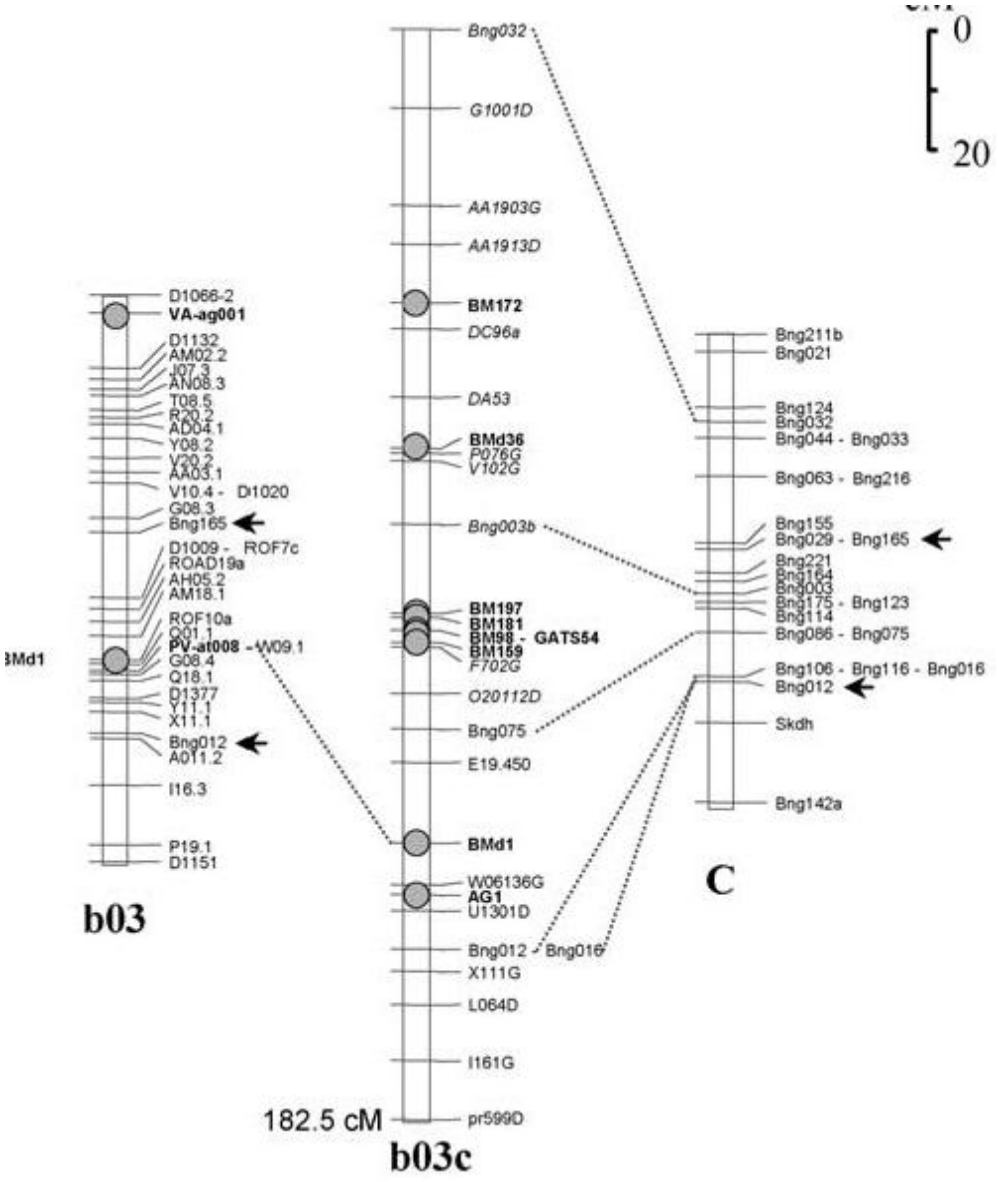
B3	PVBR255	177	GCC CTT AAC GTT GGA GTC AA TTG CCC CTT TCA GAT AAA CG
B5	BM138	203	TGT CCC TAA GAA CGA ATA TGG AAT C GAA TCA AGC AAC CTT GGA TCA TAA C
B2	BM139	115	TTA GCA ATA CCG CCA TGA GAG ACT GTA GCT CAA ACA GGG CAC
B2	BM142	157	TTC CGC TGA TTG GAT ATT AGA G AGC CCG TTC CTT CGT TTA G
B5	BM155	114	GTT CAT GTT TGT TTG ACA GTT CA CAG AAG TTA GTG TTG GTT TGA TAC A
B2	BM156	267	CTT GTT CCA CCT CCC ATC ATA GC TGC TTG CAT CTC AGC CAG AAT C
B3	BM159	198	GGT GCT GTT GCT GCT GTT AT GGG AGA TGT GGT AAG ATA ATG AAA
B3	BM172	107	CTG TAG CTC AAA CAG GGC ACT GCA ATA CCG CCA TGA GAG AT
B5	BM175	170	CAA CAG TTA AAG GTC GTC AAA TT CCA CTC TTA GCA TCA ACT GGA
B3	BM197	201	TGG ACT GGT CGA TAC GAA GC CCC AGA AGA TTG AGA ACA CCA C

Appendix 3: Linkage group B2.



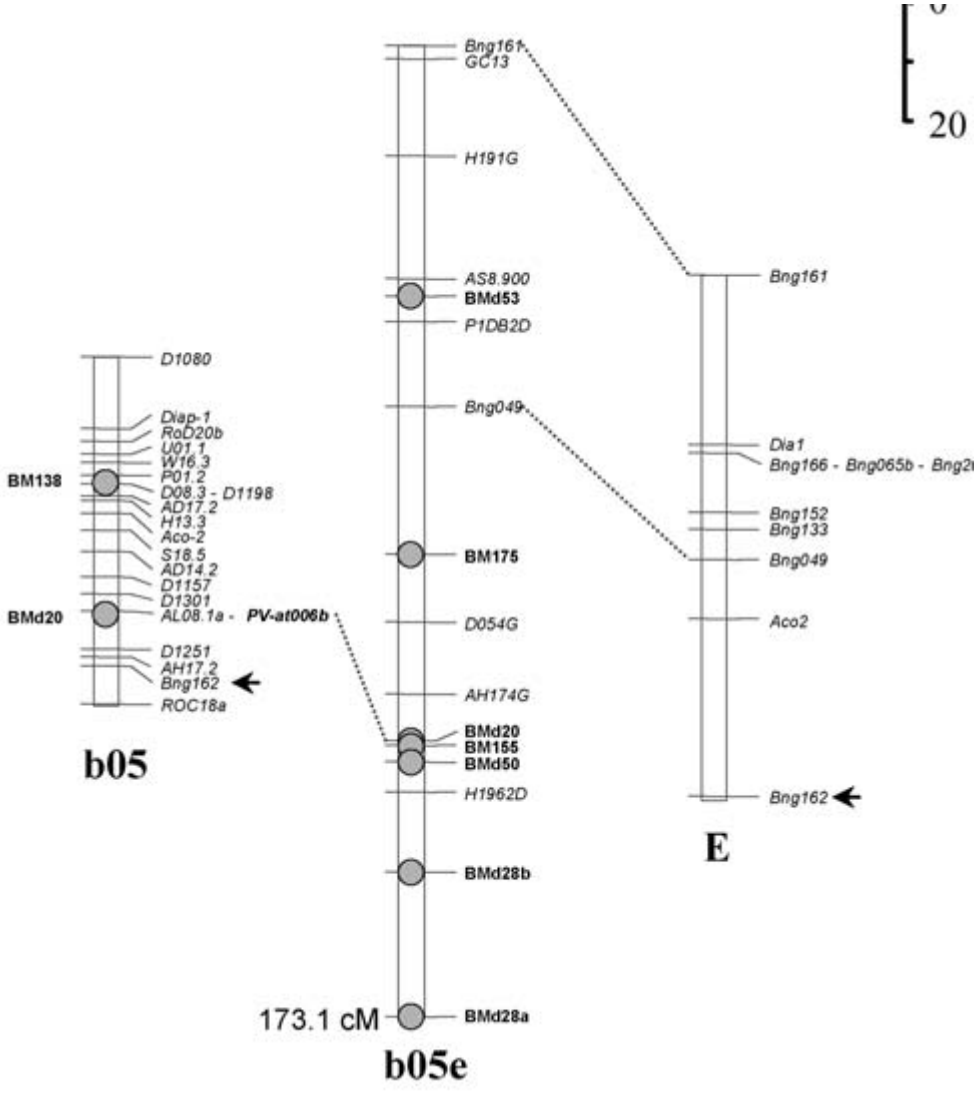
Source: Blair *et al*., 2003.

Appendix 4: Linkage group B3.



Source: Blair *et al*., 2003.

Appendix 5: Linkage group B5.



Source: Blair *et al*., 2003.

Appendix 6: Single Marker analysis using *t*-test: 62 $F_{4:5}$ lines of K132 x MLB-49-89A scored for 3 SSR markers that were not assigned to any linkage group and could not be analysed using QTL Cartographer

Marker	Difference between two means	*t*-value
BM139	0.63	0.92^{ns}
BM167	0.01	0.02^{ns}
BM172	0.56	0.86^{ns}

[c]Difference between the phenotypic means of the two groups of lines: the group carrying the susceptible parent allele minus the group carrying the resistant parent allele
[d]*t*-value statistic (Zar, 1998). The statistic tests association between markers and *Fusarium* root rot scores
[ns]Non-Significant at the 5% level of significance.